Exploring the multidimensional nature of stock structure: a case study on herring dynamics in a transition area

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PhD Thesis by

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Preface

This PhD thesis consists of a series of 7 papers and a synopsis. The papers listed below are included as Chapters within the thesis. All papers are included by permission of the respective scientific journals.

1. Clausen, L.A.W., Bekkevold, D., Hatfield, E.M.C. and Mosegaard, H. 2007. Application and validation of otolith microstructure as stock identifier in mixed Atlantic herring (Clupea harengus) stocks in the North Sea and western Baltic. ICES Journal of Marine Science, 64, 1-9

2. Bekkevold D, André C, Dahlgren TG, Mariani S, Clausen LAW, Torstensen E, Mosegaard H, Carvalho GR, Christensen TB, Norlinder E, Ruzzante DE. 2005. Environmental correlates of population differentiation in Atlantic herring. Evolution 59(12) 2656–2668.

3. Ruzzante, D.E., S. Mariani, D. Bekkevold, C. Andre´, H. Mosegaard, L. A. W. Clausen, T. G. Dahlgren, W. F. Hutchinson, E. M. C. Hatfield, E. Torstensen, J. Brigham, E. J. Simmonds, L. Laikre, L. C. Larsson, R. J. M. Stet, N. Ryman, and G. R. Carvalho. 2005. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Prog.Roy.Soc.B 273 (1593) 1459-1464

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5. Bekkevold, D., L.A.W. Clausen, S. Mariani, C. André, E.M.C. Hatfield, E.Torstensen, N.Ryman, G.R. Carvalho, D.E. Ruzzante. 2011. Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area. Mar.Ecol Prog Ser, 442: 187-199

6. Lotte Worsøe Clausen*, Karl-Johan Stæhr, Anna Rindorf and Henrik Mosegaard: Effect of spatial differences in growth on distribution of seasonally co-occurring herring (*Clupea harengus*) stocks. Submitted to Fisheries Biology January 2014

7. Lotte Worsøe Clausen*, Dorte Bekkevold*, Henrik Mosegaard, Anders Nielsen, Karl-Johan Stæhr, Tomas Gröhsler and J. Rasmus Nielsen: Rügen herring migration patterns in the Western Baltic and adjacent areas; can combined historical multidisciplinary data tell the story? Draft manuscript, submission intended to ICES Journal of Marine Science February 2014

Lotte A. Worsøe Clausen,

Charlottenlund, January 2014

Summary

Fish are not just fish. Differences within marine fish species in terms of morphology, behaviour, life history and certainly also genetic differentiation have been shown for an impressive number of species, including herring (*Clupea harengus*). These differences persist despite marine fish usually occupy areas without much environmental structuring and extensive mixing between populations occur. Many species of marine fishes have the capacity of dispersing over vast geographical areas, either passively by drifting eggs and larvae following ocean currents, or actively by migration of juveniles and adults, however, even for highly migratory species, significant population structure have been documented. Thus population structures are maintained despite extensive mixing of populations across vast distances; however the structuring factors are not easily disentangled. The factors behind this structure of populations have in some cases been referred to as spatial distance between populations, when the distribution of the species is larger than the dispersal range of individuals. Also oceanographic processes and the topography of the ocean floor have been linked to population structure in a number of species, yet few studies have tested specifically for relationships between environmental parameters of adaptive significance and population structuring in marine migratory fish, and even fewer have examined evidence of local adaptation. The relative roles of migratory behaviour and local differences in environmentally induced selective pressures in effecting such structure remain elusive. Maintaining population structures is of vital importance for the resilience of fish populations to changes in the environment and their exploitation. The preservation of intraspecific population integrity is a prerequisite for maintaining population and life history diversity which in turn affect the performance of individual species in providing important ecosystem services.

In this PhD thesis, I explore the population complexity of the herring stock called the Western Baltic Spring Spawning herring; localized in the transition area between the North Sea and the Baltic. I analyse which herring populations that are available to a mixed herring fishery in the area and their spatial and temporal occurrence. I explore the potential structuring factors causing the population diversity in the area and discuss the mechanisms behind these structuring factors. The results in this present thesis contribute to the understanding of the dynamics of the herring populations in the mixed pool of herring in the transition area between the North Sea and the Baltic. I identify several genetically different herring populations which are available for a fishery; their occurrence is structured by divergent migration strategies driven primarily by growth potential and the persistence of a genetic population differentiation is linked to the environmental heterogeneity in terms of salinity facilitating homing to spawning site. Such insight will aid a sustainable aggregated management of a fishery on a mixed herring stock. It will facilitate protecting the weaker populations from over harvesting in a mixed fishery and thus maintain the diversity and in turn the resilience of the stock to a fishery.

Dansk resumé

Fisk er ikke bare fisk. Forskelle inden for marine fiskearter i form af morfologi, adfærd, livshistorie og genetik er blevet vist for et stort antal arter, bl.a. sild (*Clupea harengus*). Disse forskelle opretholdes på trods af marine fisk normalt befinder sig områder med begrænset miljømæssig strukturering. Mange marine fiskearter har udbredelse over store geografiske områder, enten passivt ved drift af æg og larver via havstrømme, eller aktivt ved migration af unge og voksne fisk. Men selv for stærkt vandrende arter er betydelig populationsstruktur blevet dokumenteret. Disse populationsstrukturer opretholdes trods omfattende blanding af populationer på tværs af store afstande. Dog er de strukturerende faktorer ikke umiddelbart lette at identificere. De strukturerende faktorer er i nogle tilfælde blevet defineret som geografisk afstand mellem populationer, dvs. når fordelingen arten er større end hvad enkeltindivider kan vandre. Også oceanografiske processer og havbundens topografi har været afgørende for populationsstrukturen hos en række arter. Kun få studier har testet sammenhængen mellem miljøparametres adaptive betydning og populationsstrukturering i marine vandrende fisk, og endnu færre har undersøgt effekten af lokal tilpasning. Indflydelsen af vandringsmønstre og tilpasning til nærmiljø for enkelte populationer, på populationsstrukturen er endnu uafklaret. Fastholdelsen af en forskelligartet populationsstruktur og hermed mangfoldighed, er af afgørende betydning for fiskebestandenes evne til at overleve ændringer i miljøet og udnyttelsen af dem i et fiskeri.

I denne ph.d.-afhandling, udforsker jeg populations kompleksiteten af den sildebestand der kaldes den 'vestlige Østersøs vårgydende sild', som findes i farvandene mellem Nordsøen og Østersøen. Jeg analyserer, hvilke sildepopulationer, der er til rådighed for et blandet sildefiskeri i området og deres rumlige og tidsmæssige forekomst. Jeg undersøger de potentielle strukturerende faktorer, som forårsager en forskelligartet populationsstruktur i området og diskuterer mekanismerne bag de strukturerende faktorer. Resultaterne i nærværende afhandling bidrager til forståelsen af dynamikken i de sildepopulationer, der kan fanges i området mellem Nordsøen og Østersøen. Jeg identificerer flere, genetisk, forskellige bestande af sild, der er tilgængelige for et fiskeri, og viser at deres forekomst er struktureret af divergerende migrationsstrategier. Disse er primært drevet af vækstpotentiale og en lokal tilpasning af de enkelte populationer til salinitet på de respektive gydepladser. Mine resultater kan facilitere en bæredygtig forvaltning af fiskeriet på den blandede sildebestand. Det vil gøre det lettere at beskytte en svag population mod overfiskeri i et blandet fiskeri, og dermed bevare den samlede sildebestands mangfoldighed og modstandskraft overfor ændringer i miljøet eller fiskeritryk.

Chapter 1; Introduction

Fish are not just fish. There is vast documentation of differences within marine fish species in terms of morphology, behaviour, life history and certainly also genetic differentiation and the research field focusing on identifying such differences is continuously developing (Cadrin et al., 2014). Thus the long held view that abundant and widely distributed marine fish are unlikely to exhibit significant genetic structure at any but the largest of geographic scales has been shown to be largely inaccurate (e.g., Ruzzante et al. 1996; Nielsen et al. 2003; 2004; Zardoya et al. 2004). Many species of marine fishes have the capacity of dispersing over vast geographical areas, either passively by drifting eggs and larvae following ocean currents, or actively by migration of juveniles and adults, however, even for highly migratory species, significant population structure have been documented (Jørstad et al. 1991; Hutchinson et al. 2001; Mariani et al., 2005). Structure has in some cases been explained by ‘‘isolation by distance’’ (Wright 1943), which may occur when the distribution of the species is larger than the dispersal range of individuals. Isolation by distance is evident in a number of pelagic fishes and crustaceans (e.g., Palumbi 1994; Pogson et al. 2001) but not in others (Borsa 2002; Hoarau et al. 2002; Knutsen et al. 2003). Oceanographic processes and the topography of the ocean floor have been linked to population structure in a number of species (e.g., Ruzzante et al. 1998; Norris 2000; Knutsen et al. 2004), yet few studies have tested specifically for relationships between environmental parameters of adaptive significance and population structuring in marine migratory fish, and even fewer have examined evidence of local adaptation (Yamahira and Conover 2002). The relative roles of migratory behavior and local differences in environmentally induced selective pressures in effecting such structure remain elusive.

Thus population structures are maintained despite extensive mixing of populations across vast distances; however the structuring factors are not easily disentangled. The preservation of intraspecific population integrity is a prerequisite for maintaining population and life history diversity which in turn affect the performance of individual species in providing important ecosystem services (Schindler et al., 2010). A fish population complex composed of several populations appear more stable in terms of production (recruitment as a whole) because of the complementary or independent dynamics among the populations within the population complex – the so-called portfolio effect (Schindler et al., 2010). The resilience of a population complex (or stock, see box 1) is weakened if the population diversity within the population complex is decreased and the dynamics of the populations become more synchronous (Hilborn et al. 2003; Schindler et al., 2010). Stability occurs when the populations within the population complex can respond asynchronously to the same environmental conditions. Differences in life histories are important for stability, because they lead to differential responses to inter annual variations in environmental conditions (Hilborn et al., 2003, Secor et al., 2009). Preserving genetic diversity (coding such differences in life histories), and thus managing according to the evolutionary population concept, protecting the weaker populations from over harvesting in a mixed fishery,

is thus required to maintain the diversity (Schindler et al., 2010). A prerequisite for such ‘genetic diversity preservation management’ is a thorough knowledge of the populations and their vital rates; whether they are reproductively isolated from one another or whether straying between populations exists need to be considered; and knowledge of the migration patterns and potential mix between the populations within a stock is necessary (Stephenson et al., 2009). In an aggregated management in which a population complex is managed as a single population, extinction of subpopulations would be possible before the analyses of aggregated data would indicate a population decline (Frank and Brickman 2000).

Thus fish are not just fish and population complexity is structured by various factors, which neither are fully understood nor disclosed. The challenge is that investigation of population structure is a never-ending scientific endeavor that is supported by rapidly advancing technologies and methods; yet, resource conservation and fishery management require the practical definition of spatial management units that are based on the best available science and over time scales that are defined by policy. Thus the aim of population complexity research must be to provide insight of complex biological structures both to understand them in their own right and to guide their preservation.

Framing the research question: when making herring soup, does the stock matter?

The International Council for the Exploration of the Sea (ICES) defines a ‘stock’ as (a part of) a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery; in theory, a ’stock’ comprises all the individuals of fish in an area, which are part of the same reproductive process . This definition resembles definitions of the unit ‘population’, which are diverse, but all centres around a population being a group of conspecifics that typically occupy a defined geographical region and exhibit reproductive continuity from generation to generation (Waples and Gaggiotti 2006). Depending on the approach, ‘population’ is used as either an ‘ecological’ entity mainly reflecting the geographical boundary of a specific fish assemblage, or in an ‘evolutionary’ context, where designation is based on an observation of genetic structure among specific fish assemblages. Fisheries assessment tools are mainly based on the ecological criterion of populations; the corresponding management, however, is predominantly linked to the evolutionary population (Reiss et al., 2009). This mis-match does challenge the sustainable management of stocks that exhibit a high degree of complexity (i.e. a number of populations). A management ‘stock’ can be a population or part of a population of fish that maintains and sustains itself over time in a definable area or even a number of populations, which to some extent have the same life history strategy, managed as one unit stock. Throughout, the terms ‘stock’ is used in the management context and ‘population’ defining a reproductive ‘unit’ of herring in the evolutionary context; see Box 1 for a detailed categorisation.

Atlantic herring (*Clupea harengus* L.) is an abundant and widely distributed marine pelagic fish. Most herring populations are migratory and often congregate on common feeding and wintering grounds where aggregations may consist of mixtures of individuals from several populations. Herring are renowned for their plasticity challenging population definitions (Geffen 2009) and population delimitation has been intensively studied. The complexity inherent in the plasticity displayed by herring in terms of life-history strategies like migration patterns and spawning time and the highly complex population structure challenge the standard concept of 'a herring stock' within a geographical area such as a management unit . This plasticity and the well documented available methodology for stock separation makes herring an excellent object for a study of the dynamics of mixed stocks, what may drive and uphold this mixing, and the implications for management.

Definitions used here:

Population: *group of herring that occupy a defined geographical region and exhibit reproductive coherence and continuity from generation to generation. Spawning time tends to remain constant within populations over generations, but may in some cases shift among (parts of) cohorts*

Metapopulation: *group of populations in a defined geographical area exhibiting reproductive coherence within populations though allowing for some level of straying between populations*

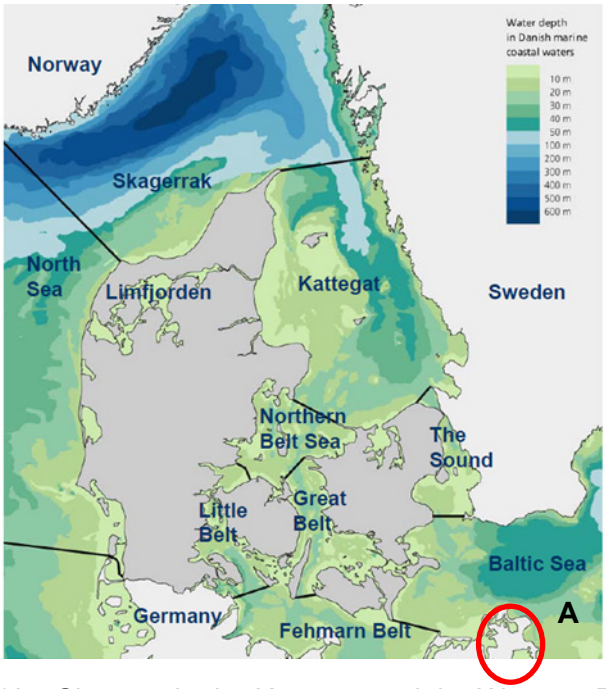
Stock: *group of herring populations with similar life history parameters that occupy a defined geographical region and are subject to a specific fishery.*

To fully assess the consequences of potentially variable occurrences of herring in a mixed fishery, the dynamics of the mixed herring populations must be described in space and time. This thesis therefore aims to disentangle the jumble of herring population occurrences over a seasonal cycle in a case study of herring occurring in an area known to be subjected to a mixed herring fishery. **The question asked is which herring are available for a fishery when and where during a year and what may determine any structure of herring occurrences found?**

The case study: Herring in the Western Baltic, the Kattegat and the Skagerrak.

To gauge the relative importance of various potential mechanisms for population structure in marine pelagic fishes it is useful to combine information about levels of gene flow obtained from neutral genetic markers with knowledge about migratory behavior and environmental factors that are likely to be of adaptive significance. Atlantic herring from the North Sea–Baltic Sea transition zone provide a model system for comparing patterns of migration and gene flow and

for testing hypotheses of environmentally induced barriers to gene flow in a highly migratory pelagic fish. In this area, mixed feeding aggregations generally comprise herring from the North Sea and from the area spanning the transition zone between the North Sea and the Baltic Sea proper (here collectively referred to as the 'Western Baltic Sea'). The Skagerrak-Kattegat-Western Baltic makes up the transition area between the brackish Baltic Sea and the North Sea (Figure 1). This transition zone represents a major environmental gradient, as salinity changes from a full marine scale at 35‰ in the North Sea to almost freshwater in the inner parts of the Baltic Sea. Temperatures also differ substantially. The North Sea confers a more stable thermal environment whereas the Baltic with its shallower waters is subject to large annual variation. The change in system structure happens gradually throughout the area and the separate units (the Skagerrak, the Kattegat and the Western Baltic). Notably, these changes in system temperature and salinity characteristics happen gradually throughout the area and the separate units (the Skagerrak, the Kattegat and the Western Baltic) have distinctly different hydrographical



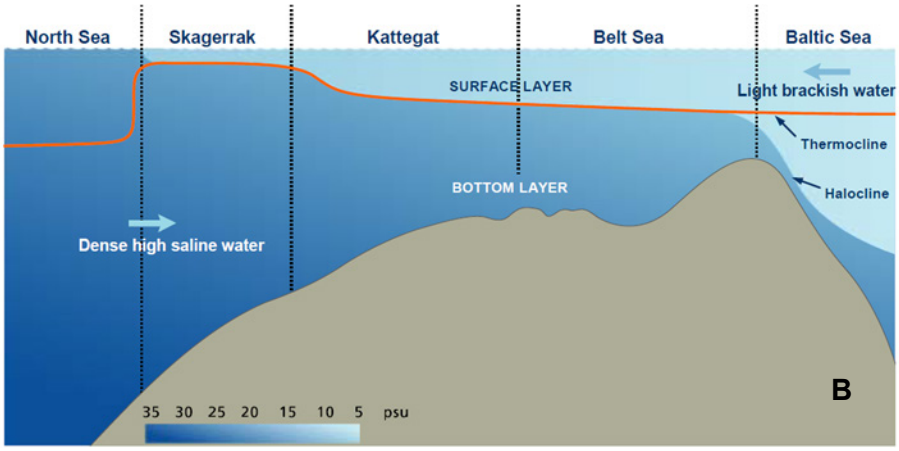


Figure 1: The Transition Area between Baltic and North Sea. A: Study area with depth distributions. B: North-south cross section through the Danish waters (below) illustrating typical stratification patterns during summer. Rügen indicated by red circle. Modified from Behrens 2007

and ecological characteristics. The hydrographical and geographical characteristics of the transitional area of Skagerrak-Kattegat and the Danish waters ramified by islands and fjords in combination with the archipelago and bays on the Swedish side (Figure 1), have given rise to a

number of more or less separate herring populations in the Skagerrak-Kattegat-Western Baltic; historically no less than 25 such stocks have been recorded (Poulsen 1975). Herring has been a very important commercial species in the Study region since Medieval times (von Dorrien et al., 2013; Klinkhardt 1996) and given this commercial value, records of herring occurrences and dynamics hereof have been made for decades.

Herring are known to display large fluctuations in population size, e.g. the Bohuslän herring periods (Alheit and Hagen 1996), the North Sea autumn spawning herring (Dickey-Collas et al., 2010) and the Norwegian spring spawning herring (Slotte 1999). In the Western Baltic, the autumn spawning herring were the dominant commercial fishery population during the first half of the 19th century (Poulsen 1936) and supporting a large fishery until the sixties of the last century (Rechlin and Borrmann 1980). A strong decrease in the occurrence of local autumn spawning herring in the Kattegat and Western Baltic has been observed since the 1940's and since 1980 likewise a decrease of local winter spawning herring in the Sound and the southernmost parts of Kattegat (Otterlind 1987). In recent times, the area is home for two herring stocks, that either spawn and use the area as nursery or migrate through it: North Sea Autumn spawning herring (NSAS) and Western Baltic Spring spawning herring (WBSS), the latter being composed of a rather large complex of herring populations from the Skagerrak, Kattegat and Inner Danish waters, and Western Baltic populations (e.g. Rügen, Schlei and Flensburg). Very recently a part of the Central Baltic herring population has been documented in the Western Baltic area, apparently using is as a summer feeding area (Gröhsler et al., 2013).

Thus the management of the herring fishery in the Skagerrak-Kattegat and Western Baltic is challenged with complex dynamics of several herring populations with different migration patterns and population dynamics. The dynamics of the mixed herring populations (and in turn stocks) in the area have never been fully described in space and time nor have the structuring factors behind the occurrence of these herring populations been examined. Through a series of Chapters, this thesis aims to disentangle the biocomplexity of herring in the transition area. First, the herring occurrences must first be identified (Chapter 2), and then any spatial structure over a full year-cycle for the identified herring populations must be examined (Chapter 3). Following this it can be explored what may determine such structure (Chapter 4). This introductory chapter briefly gives the background for each Chapter in the thesis, outlining the questions and approaches used to answer the overall question of the thesis which is synthesised in the concluding Chapter 5.

Defining herring occurrences in the Study area; introducing Chapter 2

Historically, the herring in the Skagerrak, Kattegat and Western Baltic have been classified as spring, autumn or winter spawners according to their particular spawning time ('spawning mode') (Biester 1979, Jensen 1949; Otterlind 1987) or based on differences in morphological characters (Poulsen 1975; Rosenberg and Palmén 1981). Analyses of mixed-feeding schools of

herring in the Skagerrak has shown that population affiliation often varies among herring present in these mixed aggregations (Rosenberg & Palmén 1981, Hulme 1995), and also for other areas (e.g. in the Norwegian Sea: Husebø et al. 2005; the North Sea: Cushing 1967; west of the British Isles: Brophy & Danilowicz 2002, 2003; and Gulf of St Lawrence: McQuinn 1997a).

Knowledge of stock structure is vital for setting appropriate management regulations in fisheries where multiple stocks are exploited in one area or fishery (Ricker, 1981). Fisheries management regimes that disregard or misidentify stock structures (i.e. patterns of genetic and life-history differences) among migratory populations that mix either seasonally or during specific life stages, have the potential to result in erosion of the biodiversity. Stock identification is thus an integral part of fish stock assessment and it is an evolving, multidisciplinary field encompassing many techniques (Cadrin et al., 2005). When one has settled on the definition of a stock for management purposes (Box 1) and has described what biological characters that are associated with this stock, then an identification of suitable stock identification methods can begin. The basic premise behind using any stock identification method is to apply a marker, which will be temporally stable. When selecting markers for stock identification it is imperative to acknowledge that markers result from processes that range in scale from ecological to generational to evolutionary depending on which marker that is applied. Markers used without an understanding of their 'behaviour' and inheritance under varying conditions can lead to false results (Booke 1999). Thus any stock identification marker must be established in a baseline returning validated stock affiliation results and then tested on mixtures of stocks to test the statistical power of the marker applied.

Morphometric analysis, analysis of calcified structures, and analysis of the genetic composition are among the most widespread tools available for stock identification of bony fish (Cadrin et al., 2005). Herring is highly studied model fish within stock identification and a wide variety of methods have been used to separate herring into populations or stocks. A prevailing method is to relate population affiliation to spawning time of the individual herring (Cushing 1967; Otterlind 1987) and the current stock splitting procedure applied in the herring assessment working group in ICES (ICES 2013) assigns individual herring to stock based on spawning time. Given the plasticity in herring in relation to growth and life history strategies (Geffen 2009), the appropriateness of such a stock identification method can be questioned; **is spawning time of the individual herring able to disentangle the mixture of populations encountered in the Study area?**

Chapter 2 addresses this question, first by analysing the appropriateness of using spawning time as inferred from otolith microstructure as a stock identification method (**paper 1**). Analysis of otolith microstructure is a powerful tool for determining life history of the individual fish; the formation of otolith microstructure is controlled by an endogenous circadian rhythm of increment formation linked to growth of the individual fish. The micro-increments are entrained by

photoperiod, but susceptible to modification by other cyclic environmental variables (Campana and Nielson 1985). Determination of hatch season and larval ambient environment are used as the key proxies for individual population affiliation for many fish species. Differences in otolith growth trajectories between herring larvae experiencing different temperature and feeding regimes have been identified in both field and laboratory studies (Moksness, 1992; Fossum and Moksness, 1993; Stenevik et al., 1996; Folkvord et al., 1997). Otolith microstructure has been used to identify larvae from NSAS and NSS stocks (Moksness and Fossum, 1991), and differences in the larval otolith microstructure have been identified in adult herring (Zhang and Moksness, 1993), and used successfully to separate adult herring from NSAS and WBSS stocks at an individual level (Mosegaard and Madsen, 1996; ICES, 2013b). Using otolith microstructure to determine stock affiliation (Mosegaard and Madsen, 1996) is based on the assumption that herring hatched in a specific season also spawn in that season (known as spawning time fidelity). However, observations of individuals spawning in a different season than that indicated by their hatch type, autumn spawning on traditional spring-spawning sites and the sympatric existence of herring with different spawning times (Brophy and Danilowicz, 2002, 2003; Husebø et al., 2005) made a revisit of these assumptions appropriate.

Given the questioning of the currently applied methodology to separate catches comprised of herring with mixed origins, alternative methodologies were explored. Referring to the plasticity of life history strategy of herring, the genetic differentiation between the herring populations in the area was analysed to find out if **the genetic differentiation between the herring populations in the Study area is strong enough to be used as a stock identification method?**

Genetic techniques used in stock identification include analyses of mitochondrial and nuclear DNA (reviewed in Cadrin et al., 2005). Applying population genetic analyses based on neutral molecular marker data have found widespread use for identification of marine fish populations the over the last couple of decades (Hauser & Carvalho 2008). Recently, high-resolution, genomic markers such as Single Nucleotide Polymorphisms, SNPs, have been shown to provide improved accuracy in genetic stock identification (Helyar et al. 2012; Limborg et al. 2012; Nielsen et al. 2012).

Genetic marker analysis has demonstrated that herring stocks can be separated and their migratory behaviour assessed using genetic markers as a ‘tag’ (e.g. Gaggiotti et al., 2009). Within the last decade a series of population genetic studies have established robust large-scale trends about genetic differences among herring spawning in different parts of the Atlantic (Mariani et al., 2005; Jørgensen et al. 2005; Larsson et al. 2007; Limborg et al., 2012; Nielsen et al., 2012). **Paper 2** examine temporally stable differentiation in highly polymorphic microsatellite DNA markers among spawning locations for herring in the Study area, with particular focus on potential structuring factors in the study area.

Characterising the mixture of herring in the Study area; introducing Chapter 3

Having examined the **'which herring'** part of the overall question of this thesis, the **'when and where'** parts can be examined by tracing the origins of the herring in samples taken from either commercial fishery or scientific samples over a year within the Case study area.

It is well known that fish populations migrate either in response to spawning, feeding, or even predator avoidance (Harden Jones, 1968). The extent and timing of these often annual migrations are generally both consistent and predictable within a given population suggesting an evolutionary advantage (Campana et al., 1999), even if a part of the migration behaviour is non-active (e.g. drift). For most marine fishes it is not fully understood how selection of migration route and destination is related to increased fitness. Atlantic herring have been intensively studied in terms of migrations, life-history strategies, and population dynamics (Dickey-Collas et al., 2009) and the literature on herring migrations is extensive starting almost 100 years ago (reviewed in Harden-Jones, 1968 and in Hay et al., 2001). Herring populations are often highly migratory with migration distance varying from less than 100 km to more than 1000 km (Slotte, 1999) and herring found on both sides of the Atlantic perform migrations among spawning, overwintering, and summer feeding areas (Hay et al., 2001). Extensive material, including tagging experiments, have shown that herring display diverse migration patterns depending on area and population, but also within the respective populations different migration behaviours have been described (Slotte 2001; Silva et al., 2013).

The relatively high population differentiation of herring results in life-history differences (spawning season and spawning location) among herring in the Case Study area. These populations have been reported as having profoundly different habitat use, some being resident all year and some only using the area as feeding habitat during summer. The dominating population, the Rügen herring, is described to spawn in spring around the island of Rügen in the western Baltic and migrate annually to the Skagerrak to feed (Biester 1979, Aro 1989), and then a number of relatively small populations spawning in a wide variety of fjords along the coastlines of the Study area (in the Kattegat and inner Danish waters, and the Skagerrak (Rosenberg & Palmén 1982)). The migration behaviour of the latter populations is understood as being very different from the Rügen herring as these populations were assumed not to perform distinct summer-feeding migrations but to remain relatively locally (Rosenberg and Palmén 1982; Otterlind 1987).

The temporal variation of the mixing of herring in the area is not fully described or quantified (ICES 2013b). Having the ability to disentangle the population affiliation at a highly detailed level, sampling the composition of the herring encountered in the Study area over time have the potential for determining spatially and temporally explicit migratory behaviour and thus achieving a higher understanding of what may structure the extent of mixture between herring populations.

Thus to get a more detailed impression of the herring mixture in the Study area, it is necessary to know whether **the mixing of herring populations is complete at all times or structured by season and/or area due to divergent migration patterns?**

To answer this, one ideally need to sample more or less continuously across time and space in the entire Study area, which is highly ambitious and unrealistic in terms of available sampling means, thus a sub-set of the Study area was chosen. Given the historical knowledge of the Skagerrak being an area where herring catches are comprised of a mixture of populations, the papers in Chapter 3 use samples taken in the Skagerrak to analyse the composition of the herring population mixtures. In **paper 3** a number of herring populations are defined based on the makers identified in previous studies (**paper 2** and Mariani et al., 2005) in analyses of samples taken during summer and winter in the Skagerrak. The analyses were performed on pooled samples and thus returning a general impression of the summer vs. winter herring occurrences in the Skagerrak, successfully addressing the seasonality part of the question posed, however, resolving spatio-temporal aspects of contributions at a finer spatial scale was addressed in **paper 5**. Here the analysis of population mixture was targeted to determine spatial relationships of herring from the North Sea, Skagerrak, inner Danish waters, and Rügen in mixed fishery samples collected across SW-NE transects in the Skagerrak in both summer and winter, and repeated over 2 years. The results were analysed with the objective of estimating fine-scale spatial and temporal population differences in migratory behaviour and habitat use, specifically with the aim to disentangle migratory patterns of the Rügen population from those of populations from the Skagerrak, the Kattegat and inner Danish waters.

Within the Study area, the Skagerrak together with the easternmost part of the North Sea is renowned to be the most optimal feeding grounds during summer due to the composition of zooplankton (Richardson, 1985, Maar et al., 2013) and the mixture of herring in these parts during summer is reported as consisting of the larger individuals of the WBSS (ICES 2013a). Bierman et al. (2010) showed in a study of the herring components in the mixed fishery during summer in the North Sea that spatial patterns in mixing of the herring components vary greatly between years. Thus not only can the mixture of herring populations vary following a certain pattern structured by season and area, but also potentially between years. Having examined the general structuring of the mixture by season and area, the mixture of herring during summer was examined in detail, asking the question **given the reported mix of populations during summer feeding migrations is there any structuring of this mix in terms of population affinity and spatial distribution of the populations?** The observed distribution, growth and condition of herring encountered in the mixed feeding aggregations in the Eastern North Sea, the Skagerrak and the Kattegat during summer was thus investigated. Separating the major herring stocks applying the otolith microstructure methodology (Chapter 2), the distribution of NSAS and WBSS during six consecutive years of acoustic surveys, was investigated to analyse which migration mechanisms are most likely to determine the early summer distribution of the

age classes of the two major stocks. Three possible scenarios were tested; i) migration is a local adaption and as such is evolutionary fixed with no variation between years, ii) migration distance is a result of size differences and fish migrate further when size at age is high or iii) migration distance is determined by local carrying capacity, in which case migration towards attractive areas is higher when abundance is low.

The structure of the community of herring in the Study area; introducing Chapter 4

Chapter 3 seeks to identify a structuring of the distribution of the herring population in the Study area and thus now the **'which' (Chapter 2)** and **'where and when' (Chapter 3)** in relation to the make-up of the herring occurrences has been analysed. This leads to the final part of the overall question, the '**what'**, as in **what determines then the structuring of the herring occurrences in the Study area?** The herring in the area are considered part of a stock with diverse and potentially locally adapted migratory populations that overlap spatially and seasonally. Stock differences have been ascribed to a range of effects, spanning from phenotypic plasticity to reproductively isolated, locally adapted population components (reviewed in McQuinn 1997a). Natal homing behaviour in herring would allow temporal persistence of populations and ensure life-cycle closure; however, herring may not completely follow such a complete reproductive isolation between populations. Straying in spawning adult herring from tagging studies have been demonstrated for Atlantic herring (McQuinn 1997b; Brophy and Danilowich 2002, 2003) and in studies of genetic differentiation between herring populations in the North Sea, overall levels of differentiation have been reported relatively low, suggesting some level of gene flow among local spawning sites (Mariani et al., 2005).

Notwithstanding potential straying and gene flow between populations, a series of population genetic studies have established robust large-scale trends about genetic differences among herring spawning in different parts of the Atlantic including parts of the Study area within the last decade (Jørgensen et al. 2005; Larsson et al. 2007; Limborg et al., 2012; Nielsen et al., 2012). This, together with the fact that significant differences in physical traits persist between populations (Biester, 1979; Otterlind 1987; von Dorrien et al., 2013) argues for the existence of mechanisms which produce and maintain a complex population structure despite their migratory capabilities and extensive mixing of populations.. The question is which mechanisms; genetic stratification is likely determined by several mechanisms; natal homing, larval retention, possibly natural selection, or divergent migration strategies. Given that genetic differentiation can be found between the herring populations in the Study area (Chapter 2) and that the spatial-temporal mixing of these populations varies over the year indicating seasonal migration patterns facilitating natal homing (Chapter 3), then the spawning events would be central structuring mechanisms. The spawning events are characterised by both spawning time and spawning site, however, the plasticity in spawning time has been well established for herring

(Geffen 2009). An analysis of the likely origin of sympatric populations in the Study area would potentially clarify whether **spawning time is the structuring mechanism in the diversity of herring populations in the Study area?**

Two different hypotheses may explain the origin of sympatric components with divergent spawning times. The first, called 'year-class twinning' (McQuinn 1997b), entails a scenario by which juvenile growth coupled to variation in environmental conditions in some years causes fractions of individuals to mature and spawn in an earlier or later season than that in which they themselves were spawned (and hatched). Once individuals have switched they are expected to continue spawning in that season throughout their lives. In this case, seasonally separated sympatric components thus share population origin, and spawning time reflects a plastic response to external cues, operating under alternative reproductive strategies (Gross & Repka 1998). In the second scenario, temporally divergent spawning components arise through founding events from populations exhibiting a different, be it genetically or environmentally determined, spawning season. In this scenario, sympatric components have different evolutionary origins and are expected to display genetic differentiation. Thus to determine whether the spawning time is genetically fixed and thus a structuring factor of the herring populations in the Study area, the genetic relationships and the most likely origin of two western Baltic winter spawning components that occur sympatrically with larger spring-spawning components was examined in **paper 4**.

In the Study area the spawning sites are found along most of the coastline in the area (Figure 1) in shallow, semi-enclosed areas (estuaries, lagoons) characterised by steeper slopes, exposed to currents (von Dorrien et al., 2013; Otterlind 1987). Given the variation in salinity, the spawning sites are thus characterised by a variable environment in terms of salinity and temperature throughout the Study area (Figure 1). If significant genetic structuring is evident across the strong environmental cline separating the fully marine North Sea from the brackish Baltic Sea as analysed in Chapter 2 and indicated within the Baltic Sea (Jørgensen et al. 2005), then the characteristics of the spawning sites themselves may be a structuring mechanism too. In a study of the spatial and genomic scales at which herring populations are likely to exhibit adaptation to local environments, Limborg et al (2012) found that functional genes were triggered by salinity more than temperature. This may imply that environmental heterogeneity is an important driving force of divergent selection among populations, even in high gene flow organisms. Looking further into the genetic differentiation of the herring in the Study area with particular focus on environmental correlates (**paper 2**), the structuring mechanism of the diverse environment in the Study area can be further explored and lead to a clarification of whether **plasticity in spawning time makes the spawning site a structuring factor, structure being related to the salinity profile of the Study area?**

Another behavioural factor which may give rise to a structuring of populations could be

divergent migration strategies. In a study of life history adaptations in the reproductive output in populations of Norwegian Spring Spawning herring, Silva et al. 2013 hypothesise that the divergent migration patterns observed for migratory (oceanic), likely semi-stationary (coastal) and stationary parts of the overall Norwegian Spring Spawning herring stock may be genetically founded. However these different migration patterns could per se have given rise to a differentiation of the Norwegian Spring Spawning population, structuring the population into genetically different populations. To examine such a 'hen or the egg' question, one need to analyse a population known to be genetically well-defined displaying a diverse migration behaviour. The Rügen herring population in the Study area has been reported to be well-defined genetically (Limborg et al., 2013) potentially corroborated by the results from **papers 2, 3 and 5** and this herring population has historically been characterised as having a diverse migration pattern with a varying distance of the summer feeding migration (Biester, 1979; Otterlind, 1987; von Dorrien et al., 2013). Thus the Rügen herring population appears to be optimal for a study of whether divergent migration patterns can give rise to genetic structure of populations.

The Rügen herring is a spring-spawning population spawning in the Greifswalder Bodden an estuary SE of the German Rügen Island (Figure 1) (Klinkhardt, 1996). The spawning has been reported as to occur in 'waves', the older herring usually spawning first, with the younger herring and recruits spawning in subsequent weeks (Biester 1979; Klinkhardt 1996; Nielsen et al., 2000; von Dorrien et al., 2013). Results from previous traditional tagging experiments and fishery information indicate a typical migration pattern of Rügen herring between the main spawning grounds around Greifswalder Bodden and surrounding areas to feeding areas north-westward migration extending to the Kattegat/Skagerrak /North Sea area a (Aro 1989; Biester 1979; Nielsen et al. 2001; Otterlind 1984, 1987). The feeding migration is anecdotally reported not to be performed in large dense schools; such schools are however formed during the summer feeding in Skagerrak and Kattegat. The school formation is retained during the overwintering, which based on acoustic data has been reported to mainly be centred in the Southern Kattegat and the Sound (Nielsen et al., 2001). The summer feeding migration has been reported to be age-dependent, the older individuals migrating furthest from the spawning site; this is examined in Chapter 3, however, any such structure of migration need to be investigated over a full yearly circle for all age groups to reveal any structuring mechanism. Thus given the existence of spawning waves and a divergent migration pattern both claimed to be driven by age of the individuals, the question is **what structures the migration pattern of the Rügen herring over a year?** In **paper 7** the population identification markers (both genetic and otolith related) identified in Chapter 2 are applied as well as morphological data to document the migration patterns of Rügen herring. The paper examines whether any life-stage or other individual traits have an effect on the migration strategy in relation to spawning, feeding migration distance and clarify the hitherto only qualitatively described seasonal and age related pattern of the Rügen herring migration.

Thesis outline

The biocomplexity, i.e. patterns of genetic and life-history differences among migratory populations that mix seasonally is potentially high within the herring encountered in the Study area. Despite intermingling freely in large nursery, feeding and overwintering aggregations, differences associated with life-history (spawning season, spawning location, migration pattern) appear to persist. Marine conservation initiatives or fisheries management regimes that disregard or misidentify patterns of genetic and life-history differences among migratory populations that mix seasonally (i.e. intraspecific component of biocomplexity) have the potential to result in the erosion of genetic resources. To fully understand the biocomplexity the genetic population structure of the herring mixture need to be confounded and any structuring factors in terms of the spatial-temporal extent of population mixing must be described. In other words; one need to know **which herring are available for a fishery when and where during a year and what may determine any structure of herring occurrences found.** The means to answer this question have been addressed in this introductory chapter and they can be summarised to the following points, which are treated in the three following chapters in this thesis:

Chapter 2: Which herring. This chapter review and identify the optimal population identification methods for the herring in the Study area through paper 1 and paper 2.

Chapter 3: When and where are the herring found? The mixture of the herring populations are analysed applying the methodology defined in Chapter 2 and any structuring of the mixing in terms of time and space is examined in paper 3 and 5. Paper 6 examines in detail any structure of the summer feeding migration mixture.

Chapter 4: What determines the structure of the herring occurrences? With off-set in the previous two chapters, the potential structuring mechanisms giving rise to the population biocomplexity of herring in the Study area is examined. Paper 4 investigates the origin of sympatric populations clarifying whether spawning time and place may determine the population differentiation. Finally paper 7 examines in detail if any behavioural mechanisms in relation to spawning and migration strategy provide a structuring of the dominating herring population in the area.

In Chapter 5, the thesis contents are synthesized and discussed in a broader context

Chapter 2: Defining herring occurrences in the Study area

Which herring makes up the herring soup?

Paper 1:

Application and validation of otolith microstructure as a stock identification method in mixed Atlantic herring (Clupea harengus) stocks in the North Sea and western Baltic

L. A. W. Clausen, D. Bekkevold, E. M. C. Hatfield, and H. Mosegaard

Herring (Clupea harengus) populations with different spawning times mix in ICES Division IIIa. For stock assessment, otolith microstructure analysis is used to determine the hatching season of individuals, classifying them into hatch type spring, autumn, or winter. The currently applied method uses visual inspection of season-specific daily increment pattern for the larval period. With this method, variability in individual microstructure and a lack of correspondence between hatch and spawning time may lead to classification error. We validate the visual inspection procedure in relation to these potential sources of error. Otoliths from spawning herring were first classified blindly and the results compared with spawning season. In all, 91% of classifications corresponded, and errors represented misclassifications mainly between autumn and winter spawners. However, the estimates may be biased if hatch and spawning times differ, and an objective method of hatch-time estimation based on linear modelling was employed, enumerating unbroken series of daily increments in 0-group herring hatched in different seasons. Visual inspection and objective estimation agreed in 89% of cases, and most of the errors were explained by overlapping hatch periods. Results show that herring older than the 0-group can be classified using multiple linear regression of hatch time on median increment width.

Paper 2:

Environmental correlates of population differentiation in Atlantic herring

Dorte Bekkevold, Carl André, Thomas G.Dahlgren, Lotte A.W.Clausen, Else Torstensen, Henrik Mosegaard, Gary R.Carvalho, Tina B.Christensen, Erika Norlinder and Daniel E.Ruzzante

The marine environment is characterized by few physical barriers, and pelagic fishes commonly show high migratory potential and low, albeit in some cases statistically significant, levels of genetic divergence in neutral genetic marker analyses. However, it is not clear whether low levels of differentiation reflect spatially separated populations experiencing gene flow or shallow population histories coupled with limited random genetic drift in large, demographically isolated populations undergoing independent evolutionary processes. Using information for nine microsatellite loci in a total of 1951 fish, we analyzed genetic differentiation among Atlantic herring from eleven spawning locations distributed along a longitudinal gradient from the North Sea to the Western Baltic. Overall genetic differentiation was low (u 5 0.008) but statistically significant. The area is characterized by a dramatic shift in hydrography from the highly saline and temperature stable North Sea to the brackish Baltic Sea, where temperatures show high annual variation. We used two different methods, a novel computational geometric approach and partial Mantel correlation analysis coupled with detailed environmental information from spawning locations to show that patterns of reproductive isolation covaried with salinity differences among spawning locations, independent of their geographical distance. We show that reproductive isolation can be maintained in marine fish populations exhibiting substantial mixing during larval and adult life stages. Analyses incorporating genetic, spatial, and environmental parameters indicated that isolating mechanisms are associated with the specific salinity conditions on spawning locations.

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Application and validation of otolith microstructure as a stock identification method in mixed Atlantic herring (*Clupea harengus*) stocks in the North Sea and western Baltic

L. A. W. Clausen, D. Bekkevold, E. M. C. Hatfield, and H. Mosegaard

Clausen, L. A. W., Bekkevold, D., Hatifield, E. M. C., and Mosegard, H. 2007. Application and validation of otolith microstructure as a stock identification method in mixed Atlantic herring (*Clupea harengus*) stocks in the North Sea and western Baltic – ICES Journal of Marine Science, 64.

Herring (*Clupea harengus*) populations with different spawning times mix in ICES Division IIIa. For stock assessment, otolith microstructure analysis is used to determine the hatching season of individuals, classifying them into hatch type spring, autumn, or winter. The currently applied method uses visual inspection of season-specific daily increment pattern for the larval period. With this method, variability in individual microstructure and a lack of correspondence between hatch and spawning time may lead to classification error. We validate the visual inspection procedure in relation to these potential sources of error. Otoliths from spawning herring were first classified blindly and the results compared with spawning season. In all, 91% of classifications corresponded, and errors represented misclassifications mainly between autumn and winter spawners. However, the estimates may be biased if hatch and spawning times differ, and an objective method of hatch-time estimation based on linear modelling was employed, enumerating unbroken series of daily increments in 0-group herring hatched in different seasons. Visual inspection and objective estimation agreed in 89% of cases, and most of the errors were explained by overlapping hatch periods. Results show that herring older than the 0-group can be classified using multiple linear regression of hatch time on median increment width.

Keywords: fisheries management, herring assessment, otolith microstructure, stock identification, strayers.

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Introduction

Atlantic herring (*Clupea harengus*) population dynamics are complex, and different stocks often display variation in life history and spawning season (Jennings and Beverton, 1991; McQuinn, 1997a) as well as genetic structuring (Bekkevold *et al.*, 2005; Mariani *et al.*, 2005). Herring perform extensive seasonal migrations between spawning, feeding, and wintering areas (Slotte, 1998), and different stock components often mix on feeding and wintering grounds (Rosenberg and Palmén, 1981; Wheeler and Winters, 1984; Husebø *et al.*, 2005; Ruzzante *et al.*, 2006). Estimation of individual population contributions to these mixed stocks has attracted considerable interest for management purposes (ICES, 2005), because the preservation of complex stock structures necessitates knowledge of how migratory components of various stocks overlap spatially and seasonally.

In the North Sea, management currently recognizes two main stocks: North Sea autumn spawners (NSAS) and winter-spawning Downs herring (ICES, 2004). These populations mix on nursery and feeding grounds in the North Sea as well as in ICES Division IIIa (Cushing, 1967; Rosenberg and Palmén, 1981; Hulme, 1995; Ruzzante *et al.*, 2006). Although meristic characters such as vertebral counts and otolith microstructure to some extent differ between the two groups (Cushing and Bridger, 1966; Hulme, 1995; Mosegaard and Madsen, 1996), little genetic differentiation has been identified between stock components (Mariani *et al.*, 2005). In contrast to the North Sea stock, western Baltic spring spawners (WBSS) comprise several genetically distinct populations spawning in Divisions IIIa, IIIb, and IIIc (Bekkevold *et al.*, 2005), of which the Rügen spawning component is assumed to be the largest and other components of relatively lesser significance. Although there may be population differences (Ruzzante *et al.*, 2006), these three stocks are collectively highly migratory, and NSAS and Downs juveniles as well as adults of WBSS origin migrate into Division IIIa, where they feed in mixed stocks. The large Norwegian spring-spawning (NSS) stock spawning along the west coast of Norway also migrates extensively (Slotte, 1998). However, an extensive literature search has not produced evidence for its migration into Division IIIa (Dragesund *et al.*, 1997; Slotte, 1998; Kvamme *et al.*, 2003; Husebø *et al.*, 2005).

Analysis of otolith microstructure is a powerful tool for determining life history trajectories, determination of hatch season and larval ambient environment being the key proxies for individual population affiliation. Differences in otolith growth trajectories between herring larvae experiencing different temperature and

feeding regimes have been identified in both field and laboratory studies (Moksness, 1992; Fossum and Moksness, 1993; Stenevik *et al.*, 1996; Folkvord *et al.*, 1997). Herring larvae hatched at different times of the year in the wild, experiencing different temperature and feeding regimes, display different patterns of primary increments in their otolith. Otolith microstructure has been used to identify larvae from NSAS and NSS stocks (Moksness and Fossum, 1991), and differences in the larval otolith microstructure have been identified too in adult herring (Zhang and Moksness, 1993), and used successfully to separate adult herring from NSAS, Downs, and WBSS spawning stocks at an individual level (Mosegaard and Madsen, 1996; ICES, 2004). For Division IIIa, the ICES Herring Assessment Working Group (HAWG) for the area south of 62°N has applied splitting keys to catches to separate NSAS and Downs herring from WBSS herring. Before 1996, the splitting key used by the HAWG was calculated from a sample-based mean vertebral count. In the period 1996–2001, splitting keys were constructed using information from a combination of vertebral count and otolith microstructure methods (ICES, 2001). From 2001 on, the splitting keys have been constructed solely using otolith microstructure methods (ICES, 2004).

Otolith-based assessment of stock affiliation (Mosegaard and Madsen, 1996) is based on the assumption that herring hatched in a specific season also spawn in that season (known both as the "pure stock concept" and "spawning time fidelity"). However, observations of autumn spawning on traditional spring-spawning sites and the sympatric existence of herring with different spawning times (Brophy and Danilowicz, 2002, 2003; Husebø *et al.*, 2005; Bekkevold *et al.*, in press) make a revisit of these assumptions appropriate now. The current study was initiated to analyse variability in the otolith microstructure pattern in post-larval 0-group herring, hatched during different seasons, to achieve a validation method independent of the assumptions behind the pure stock concept. Formation of the first annual translucent ring in herring otoliths coincides with winter stagnation of growth, and the cessation of daily increment formation (Arneri *et al.*, 1998). The 0-group herring, caught during their first growth period, were chosen according to the assumption that they would exhibit an assessable unbroken series of daily increments from the period after hatching until capture. We use information from larval increment patterns to develop an independent objective validation method that combines backtracking of the date of formation of the first primary increment with measurements of microstructure increment patterns and visual inspection of the larval otolith. Primary increments formed during the larval stage in herring are daily in Norwegian spring spawners (Moksness, 1992), so allowing back-calculation of hatch date by counting the daily increments in a fish from the edge to the centre and adding an estimated initial period with no daily increments (Moksness, 1992). However, it is unknown whether this procedure is valid across populations (Geffen, 1982; Folkvord *et al.*, 2000; Fox *et al.*, 2004).

Our study assesses the currently employed routine of identifying herring from different spawning stocks at an individual level by visually inspecting the larval otolith microstructure in both 0-group and adult spawning herring, using two approaches: (i) evaluating the accuracy of visual inspection, by assessing the extent to which hatch-type classification by visually inspecting otoliths from spawning herring collected in the North Sea (representing NSAS), English Channel (Downs), and the western Baltic (WBSS) corresponds with the respective spawning season of the individual fish, and (ii) evaluating the correspondence between visual hatch-type classification and backtracked hatch date in 0-group herring sampled from a mixed stock in Division IIIa, based on a linear modelling approach that uses objectively measured and enumerated larval otolith microstructure data.

We discuss the application of the results of visually inspecting otolith microstructure as a valid stock separation method in the light of the results from a quantitative objective validation method. We also infer the accuracy of the visual inspection and natural variability of the otolith microstructure methods in terms of the ability to indicate violated assumptions of, for instance, spawning time fidelity.

Material and methods

Validation by visual inspection of spawning herring assuming spawning time fidelity

Ripe-and-running (maturity stage 6) herring were sampled from collections from spawning sites in the North Sea, English Channel, and western Baltic (Table 1, Figure 1). We assumed there were no strays from extant populations with divergent hatching and spawning times in our sample. Otoliths were mounted with the sulcus side up in thermoplastic resin (Buehler Thermoplastic Cement no. 40–8100) at 150°C to facilitate grinding and polishing of both sides. The identity of each individual was coded in such a manner that readers were unable to detect from which population the fish originated. The order of the otoliths was set so that the three possible hatch types (spring, autumn, and winter) appeared randomly. The otoliths were polished using a series of grinding and polishing films with decreasing grain size from 30 to 0.3 μm, to optimize the visual resolution at a focal plane through the otolith's nucleus and a transect from this to the edge.

Table 1. Sampling of 0-group herring and spawning fish in the years 2001–2003.

State	Sampling year	Sampling month	Sampling area	Number of individuals
0-group	2001	August	IIIaN, IIIaS	12
		September	IIIaN	13
	2002	July	IIIaS	8
		September	IIIaS	22
	2003	July	IIIaN, IIIaS	5
		November	IIIaN	25
		December	IIIaN	23
Spawning	2001	November	English Channel	40
		December	English Channel	45
	2002	March	Sub.div.24	98
		April	IIIaN	1
		August	Sub.div. IVb	146
	2003	March	Sub.div. 24	192
		April	IIIaN	1
		August	Sub.div. IVb	91
		September	Sub.div. IVb	83

Sampling areas described in Figure 1.

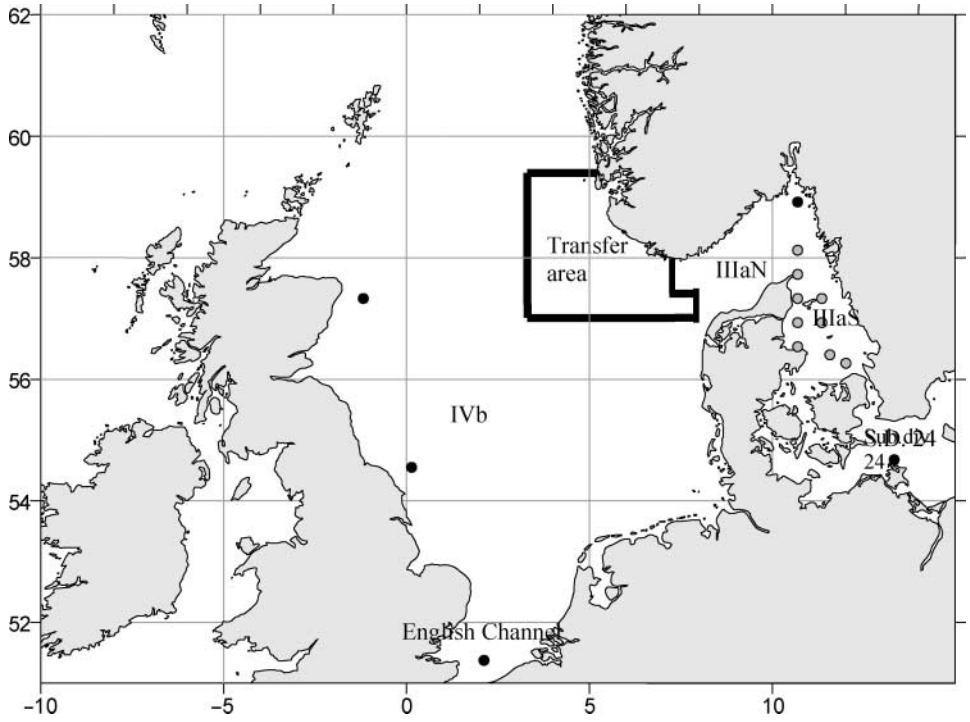

Figure 1. Sampling locations for spawning herring (black dots) and 0-group herring (grey dots). Sampling year and months are listed in Table 1.

Hatch type was estimated for all 697 herring examined. Visual inspection was performed by two experienced readers using a Leica^{TM} DMLB compound light microscope with objective lenses of 20× and 40× magnification and a long distance between focus and lens to facilitate viewing of the otolith microstructure through a microscope slide 1.5 mm thick. The readers assigned a hatch type of either spring, autumn, or winter to all otoliths by visual inspection, following the normal laboratory guidelines presented in Table 2. Through experience, the readers have been able to calibrate their perception of distances in the view field of the microscope, such that they know (approximately) the scale of measurement at the two magnifications used. The zone where incremental widths increased from <2 to >2.5 μm was used as a marker for the onset of increased spring growth conditions (Mosegaard *et al.*, 2001). Otoliths considered as unreadable by one or both readers were disregarded for that comparison. The accuracy of the visual inspection of hatch type was calculated as the correspondence between the assigned hatch type and the season in which the fish spawned, assuming that all individuals exhibited spawning time fidelity.

Table 2. General guidelines followed when determining hatch type by visual inspection. All otolith types are variable, and the appearance depends on the stock and exact timing of hatch.

Hatch type	Criteria for visual inspection of the otolith microstructure
Spring	Wide increments, rapidly increasing in width very close to the centre of the otolith Highly variable Early-hatched fish exhibit increments rapidly increasing from a width of 2 to >4 μm Later-hatched fish have relatively wide increments of about 4 μm already 20–40 μm from the nucleus
Autumn	Otolith increments <2.5 μm wide are found >200 μm from the centre All increments appear to have close-to-constant widths
Winter	Otolith increments gradual increase from about 1 μm width about 10 μm from the centre to >3 μm wide at a distance of 150 μm from the centre The increase in increment widths accelerates at about 200 μm from the centre

Validation by image analysis of otolith microstructure pattern in 0-group herring

A search in the DIFRES database on 0-group herring (fish caught before the onset of the first annual otolith winter ring) from Sub-divisions IIIaN and IIIaS and the transfer area in the North Sea (Figure 1) between 2001 and 2003 was made, and 108 fish were selected from different locations within the area (Table 1, Figure 1).

The otoliths of these herring were retrieved from archives, remounted, and inspected visually following the same procedure as described earlier for otoliths from the spawning populations. After preparation, all otoliths could be classified as autumn, winter, or spring hatch types. Following visual inspection, images of 0-group herring sagittae ($n = 108$) were digitized; each otolith was analysed by taking several pictures following the longest axis along the postrostrum. Measurements of otolith microstructure were made with a Leica 350 F digital camera and ImagePro^{TM} 5.0 image-analysis package for Windows^{TM}. Increment widths were measured automatically using the Caliper tool in ImagePro, along a profile of grey values and using a profile bandwidth of 10 μm.

The Caliper tool was set to identify the onset of an increment as the point at which the grey values changed towards lower values at the fastest rate. The process was monitored by an expert reader, and if the program produced obviously erroneous increments (e.g. because of cracks in the otolith), these were altered manually to fit the real increments. In cases where the increments were not sufficiently clear to be identified by the Caliper tool or by eye, the distance from the last visible increment to the next visible increment was measured. A minimum acceptable increment width was set at 0.5 μm, to filter out the segments where false or no daily rings were visible along the measurement axis. All measurements were transferred to an MS Excel™ spreadsheet. Areas with no detectable ring structures were occasionally found in the trajectory from the otolith centre to the edge. As these areas would appear as abnormally broad increments but only represent 1 d, a running median value $m_i = \mathrm{MED}(w_{i-2}, \ldots, w_{i+2})$ was applied as a smoother to yield a robust estimate of increment width at distance from centre. This median was then used to estimate duration in days (d_i) between observed increments w_i, as $d_i = w_i/m_i$, independent of whether these were true daily increments or just zones with several unreadable daily increments. A median over five successive increments was enough to screen out all unreadable areas. This was indicated by the fact that no median increment exceeded 7 μm in the first 200 μm from the centre, and no median at all was more than 14 μm wide. Further, only five successive pairs of medians out of 28 300 had more than a 50% change in width between them.

Initial otolith increment position after hatch in herring larvae subjected to suboptimal growth conditions (as in autumn and winter) is sometimes not discernible. Therefore, measurements closer than 10 μm from the centre were disregarded following the findings in Folkvord *et al.* (2004). In 94% of otoliths, the first measurable increment was formed at a radius (R_1) less than 25 μm from the centre. To estimate the number of increments formed in the zone between 10 μm from the centre and R_1, otolith initial growth rate was analysed as a function of day of formation. As a measure of early otolith growth rate, the median increment width of the first six measured increments (m) was regressed vs. Julian day number minus 200 at 25 μm from the centre (J) in a quadratic relationship: $m = 1.67 + 0.0081J + 0.000037J^2$ ($r^2 = 0.75$, $p < 0.0001$, $n = 108$). The value of −200 is applied to achieve the most monotonic quadratic function and gives the best display of the different seasons' growth patterns. Assuming that increments are daily, this relationship was then used to extrapolate the number of days in the unreadable zone from 10 μm to the first measurable increment [number of days $= (R_1 - 10)/m$]. The total estimated age at catch was then subtracted from the Julian day of the catch to obtain the Julian day of first possible ring formation (10 μm from the centre), which was then used as a proxy for hatch date, neglecting the possible initial period of very slow otolith growth after hatch (Folkvord *et al.*, 2004). This estimated hatch date, based on counts plus additional zones with an estimated number of increments, is hereafter referred to as the back-calculated hatch date h.

As this method is only applicable to fish with a potentially unbroken series of daily increments, e.g. 0-group herring, we analysed how shorter series (segments) of measured increment widths would estimate h. Owing to variable resolution in the segment 0–15 μm from the centre, this area was neglected in the analysis.

Starting from a distance of 15 μm from the centre, the otolith trajectory was divided into k segments of 30 μm width, and each k segment's median increment width (m_z) was used as independent variable in a multiple regression analysis ($z = 1$ for 15–45 μm; $z = 2$ for 45–75 μm, etc.). Both original and natural log-transformed values [$\ln(m_z)$] were explored. Stepwise regression analysis was performed to obtain a selection of significant coefficients from the total array of coefficients corresponding to all k segments (a, b_1, b_2, ..., b_k). The estimation of hatch date from the multiple regression may be expressed as $h = a + \sum(b_j \times f(m_j))$, where f is the untransformed or ln-transformed median increment width and j is an index of the subset of measured segments giving a significant linear combination for the estimation of hatch date h.

Summer, when very few herring have hatched, constitutes a natural separation between fish hatched in spring and fish hatched in autumn. A good separation was found by letting summer start at Julian date 189 − 365 = −176. The distribution of h was then analysed using the cumulative frequency distribution from $h = -176$ to 189. A plot of the data for the 108 herring (Figure 2) suggested the existence of three major aggregations in time (from approximately −150 to −70; −25 to 70; and 90 to 150). Assuming normal distributions of the three clusters, the number of individuals (N_k), the mean (μ_k) hatch date, and its standard deviation (σk) for each cluster (k) were estimated by the minimum sum of squares (SSQ) method:

$$\mathrm{Min(SSQ)} = \mathrm{Min}\left[\sum_{i}^{108}\left[\frac{\mathrm{Rank}(h_i)}{108} - \frac{\Phi(h_i,\mu_1,\sigma_1)\times N_1 + \Phi(h_i,\mu_2,\sigma_2)\times N_2 + \Phi(h_i,\mu_3,\sigma_3)\times N_3}{108}\right]^2\right]$$

Here, Φ is the cumulative normal distribution with estimated mean μ_k and standard deviation σ_k, and N_k is the estimated number of individual fish in the kth of the three hatch groups (autumn, winter, or spring).

A knife-edge separation of individual hatch season ($S_{aut} = 9$, $S_{win} = 12$, $S_{spr} = 16$) was calculated by the highest probability of belonging to a specific period. The ability of $f(m_z)$, from the different measured segments, to estimate hatch season S_k was explored using stepwise linear regression, with $\alpha = 0.05$ for parameters staying in the model. Classification success was compared between analyses of segments in two different otolith areas, dependent on experience in routine preparation of the larval otolith centre in adult herring: (i) when the centre remains intact with visible segments from 15 to 225 μm ($z = 1, 2, \ldots, 7$) and

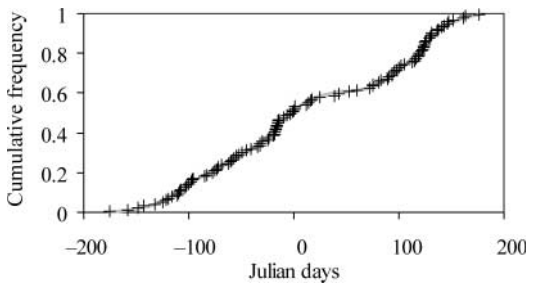

Figure 2. Cumulative distribution of back-calculated hatch dates in 0-group herring from counts of daily increments along a transect from the otolith centre to the edge (plus signs, raw data; line, cumulative sum of three estimated normal distributions).

Table 3. Accuracy of visual inspection of hatch type in spawning herring assumption spawning time fidelity.

Visual inspection	Sampling season		
	Spring (%)	Autumn (%)	Winter (%)
Spring	97	1	0
Autumn	2	92	32
Winter	1	7	68

(ii) in cases of overgrinding, where usually only increments >300 μm from the centre are visible.

Results

Validation by visual inspection of spawning herring assuming spawning time fidelity

The accuracy of visual inspection of hatch type is presented in Table 3. Assuming that herring exhibited spawning time fidelity to hatch date, the accuracy of visual inspection was high with an overall correct classification of 91%. Herring collected ripe-and-running in winter (November–December) were most difficult to classify, with a misclassification rate of 32%, whereas individuals collected in spring (March–June) were of a hatch type determined with the lowest misclassification rate of 3%. The most apparent pattern in the misclassification was that spawning herring from autumn and winter were most frequently confused with each other, whereas spring spawners were assigned equally often to winter or autumn hatch type when misclassified (Table 3).

Validation by image analysis of otolith microstructure pattern in 0-group herring

Most otoliths had whole unbroken transects of daily increments from the start of measurement (at an average 20 μm from the centre) to the edge of the otolith (95% of the otoliths had 92% of the transect complete without interruption). The distribution of back-calculated hatch dates is shown in Figure 3, in which the smooth curves are the fitted normal distributions of hatch dates based on the backtracked number of days from catch. The back-calculated hatch dates fell within three well separated groups, winter, spring, and autumn. However, some overlap between groups was evident, especially between the autumn and winter

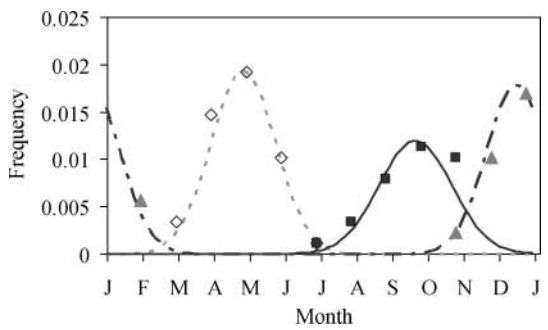

Figure 3. Back-calculated hatch date distributions of 0-group herring analysed by counts of daily increments along a transect from the otolith centre to the edge. Observed data are grouped by 30 d intervals (symbols), and the corresponding normal density distributions (lines) are estimated by minimum sum of squares based on cumulative raw data (see Figure 2) (squares, autumn; triangles, winter; diamonds, spring).

Table 4. Accuracy of visual inspection of hatch type in 0-group herring when compared with the back-calculated hatch season based on micro-increment enumeration.

Visual assigned hatch type	Back-tracked hatch season		
	Spring (%)	Autumn (%)	Winter (%)
Spring	95	0	3
Autumn	0	90	17
Winter	5	10	80

hatch date groups. The periods for the hatching seasons were defined by normal distribution, spring being from 18 February to 9 July, autumn from 9 July to 5 November, and winter from 5 November to 18 February.

Visual inspection of 0-group otoliths gave an overall correct classification of 89% when the classification by visual inspection was compared with the back-calculated hatch season of individual fish (Table 4). The misclassification pattern repeated the pattern seen in the pure stock samples, the most frequently confused hatch types being those of autumn and winter. Autumn-hatched herring were classified as winter-hatched in 10% of the fish analysed, and winter-hatched herring were classified as autumn-hatched by visual inspection in 17% of those analysed.

The large overlap between autumn and winter hatch seasons and the poor fit of the later autumn-hatched herring (Figure 3), together with the pattern of greater misclassification by visual inspection between autumn- and winter-hatched fish than between either of these two and spring-hatched fish (Table 4) led to further examination of the division between autumn and winter hatching seasons. The seasons were subjectively forced to fixed periods so that the classical start of winter was applied, categorizing winter hatch as from 1 December to 18 February, spring from 18 February to 9 July, autumn from 9 July and 5 November, and late autumn from 5 November and 1 December. Using these four categories, the visual inspection results were re-analysed, revealing that 6% of the misclassified winter hatch types fell within the period late autumn (Table 5). The subjective categorization of hatch season did not affect classification of spring hatch types, whereas classification success of winter hatch types increased.

Although three well separated hatch date groups were found, there was a significant within-group difference in mean hatch date for both autumn ($p = 0.04$) and winter ($p = 0.0018$) groups among the three sampling years (2001–2003). The development of increment width with distance from the otolith centre is shown for the four hatch types (as determined by increment counts to winter, spring, autumn, and late autumn) in Figure 4. The spring-hatched herring clearly separated from the remaining hatch types

Table 5. Accuracy of visual inspection of hatch type in 0-group herring when compared with the back-calculated hatch season with defined periods for spring, autumn, late autumn, and winter.

Visual assigned hatch type	Back-tracked hatch season			
	Spring (%)	Autumn (%)	Late autumn (%)	Winter (%)
Spring	96	0	0	4
Autumn	0	86	4	10
Winter	5	4	6	85

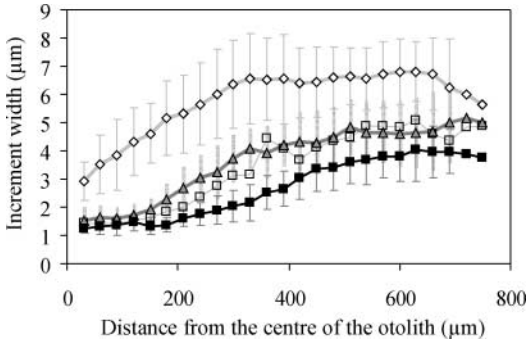

Figure 4. Development of micro-increment width in the first 750 μm from the centre of the otolith for spring-, autumn-, late-autumn-, and winter-hatched 0-group herring (bars indicating ± 1 s.d. are not given for late autumn, to enhance readability). (diamonds, spring; closed squares, autumn; open squares, late autumn; triangles, winter).

by exhibiting increments wider than 2 μm from the beginning of measurements, and the increments continued to increase in width over the whole measurement transect, levelling out at approximately 6 μm at a distance 400 μm from the centre. The increment width development in autumn-, late-autumn-, and winter-hatched herring overlapped, especially in the first part of the measurement transect. At a distance 150 μm from the centre, the three types can be separated, the late-autumn hatch type being on the line of gradual change from autumn to winter type.

To aid hatch type determination by visual inspection in these hatch types that are more difficult to separate, a series of segments along a transect from the otolith centre towards the edge was selected, and the separation ability of the increment widths in these segments were tested using a multiple regression analysis.

When median increment widths, m_z, from segments 1–7 of the otolith (i.e. the area from 15 to 225 μm from the centre) were analysed, the linear combination $S_k = 7.8 + 1.3 \times \ln(m_1) + 1.6 \times \ln(m_5) + 4.8 \times \ln(m_6) - 0.74 \times m_6$ ($r^2 = 0.88$) exhibited the best fit (Figure 5). However, a large number of other segment combinations also gave a good prediction of hatch season, with segment 5 (135–165 μm from the centre) often showing up as the major influence. When segments with $k > 10$ were analysed, the best combination was $S_k = 1.6 + 2.6 \times \ln(m_{11}) + 3.0 \times \ln(m_{14}) + 0.45 \times m_{20}$ ($r^2 = 0.76$), with segment 11 (315–345 μm from the centre) generally having the highest influence in different combinations.

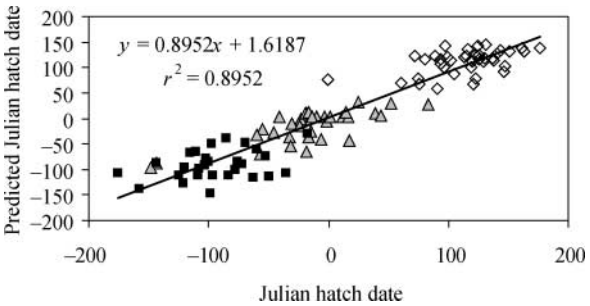

Figure 5. Relationship between back-calculated hatch date and predicted hatch date based on multiple regression analysis of otolith micro-increment measurements (m_z) from segments 1–7 of the otolith, i.e. the area from 15 to 225 μm from the centre. Fish were further assigned hatch season by visual inspection (squares, autumn; triangles, winter; diamonds, spring).

Discussion

To ensure conservation of herring population diversity in the North Atlantic, all stock components and their natural migration patterns should be considered in compiling scientific advice on the fishery (Stephenson, 2001). Therefore, obtaining high levels of precision in the input data to the assessment of mixed stocks is warranted. The good agreement between the hatch type assigned by visual inspection and the sampling season of spawning herring observed in this study confirms visual inspection of larval otolith microstructure in spawning herring as a valid method of discriminating between hatch types. However, despite the high level of correspondence between assigned hatch type and spawning season, some variation was observed among the readers' classification results. A lack of correspondence between estimated hatch and spawning season was mainly discriminating between autumn- and winter-spawned herring. An explanation for the misclassifications may be found in a possible straying of fish not exhibiting spawning time fidelity (McQuinn, 1997b; Slotte, 1998, 2001). For studies of population structuring, the identification of fish straying among populations with different spawning seasons is of focal interest, but their potential existence also presents a problem concerning validation of the otolith microstructure method. The otolith microstructure of straying fish may not be detected because the apparent variability of the otolith microstructure may be too high to allow detection of the phenomenon. If, on the other hand, a specific hatch period gives rise to highly variable otolith microstructure, some fish may be falsely identified as originating from populations exhibiting different spawning seasons. The misclassification rates observed in this work were higher when gonad stage was used as an indicator of spawning time, than when using back-tracked hatch dates (Tables 3 and 4). This could suggest some spawning season straying. However, it could also be related to a natural variability in the larval otolith microstructure formed after hatch, as well as potentially overlapping spawning seasons.

The variation between reader-assigned hatch type and gonad stage indicated that spawning time may be affected by both within- and among-reader variation, influenced perhaps by insufficient training and/or lack of quality control during the reading process. This issue was not investigated in our study. However, both readers in this study have been tested already for reader consistency and exhibit good correspondence between hatch type determination (ICES, 2005).

The quantitative objective classification method of hatch types in 0-group herring developed here provides an opportunity to calibrate the visual inspection of hatch types in all herring. Inclusion of possible variation in environmental influence on otolith microstructure allows for variability in the pattern within each hatch type. The underlying assumption for this approach is that primary increments are sufficiently close to being daily for back-calculation of hatch season in 0-group herring to be possible. A further development of the method assumes that increment measurements along radii at specific distances from the otolith core reflect season- and area-specific environmental conditions during the larval growth phase, so permitting the use of otolith microstructure patterns to identify offspring from different spawning populations.

Although it was not intended to estimate the absolute hatch date, but rather to estimate the hatch season with reasonable

accuracy, the validation technique presented here has two important prerequisites: knowledge of the timing of formation of the first daily increment and of the successive daily deposition of micro-increments in the larval otolith. The formation of the first discernible daily increment in herring larvae coincides with the onset of first feeding at the start of post-yolk-sac growth (Moksness, 1992; Høie *et al*., 1999). This takes place in herring around 10–19 d from hatching, depending on the population in question (Fox *et al.*, 2004). However, growth rate and temperature strongly influence the formation of the first discernible increment (Høie *et al*., 1997; Folkvord *et al*., 2000; Pavlov *et al*., 2000; Fox *et al*., 2004). Folkvord *et al.* (2004) found no increase in size of sagittae from herring larvae reared at 4°C up to 30 d, whereas herring reared at 12°C showed sagittal growth after 9 d. We calculated initial undetectable increment widths by a general curvilinear relationship from the fish with clearest otolith patterns. However, otolith no-growth under abnormal environmental conditions could not be detected using our methods. Adding a variable number of days to the counts of daily increments, depending on some uncertain environmental forcing, would make calculation of absolute age more uncertain than necessary for our purpose. In this study, it is likely that the ages of the winter hatch type herring were underestimated, because these fish would have experienced the lowest post-hatch temperatures of the three hatch types. However, estimates for fish hatched during autumn could also have been lower than the actual ages, depending on specific hatch time and annual variation in temperature.

The formation of daily increments in embryonic stages of herring has not been confirmed (McGurk, 1984; Moksness *et al*., 1987). However, for stages following the absorption of the yolk sac, otolith micro-increments are formed on a daily basis (McGurk, 1987; Moksness and Wespestad, 1989; Moksness and Fossum, 1991; Moksness, 1992). Growth rate, however, seems to influence the deposition of daily increments. Several studies have demonstrated non-daily increment deposition in herring larvae with a growth rate <0.4 mm d^{-1} (Geffen, 1982; McGurk, 1984; Folkvord *et al*., 2000; Pavlov *et al*., 2000; Fox *et al*., 2004). Autumn-spawned herring larvae may exhibit growth rates below this value (Munk and Christensen, 1990; Johannesen *et al*., 2000), and it is therefore likely that non-daily rates of micro-increment formation in these fish could lead to underestimating the absolute age, as seen in Feet *et al.* (2002) and Fox *et al.* (2004). Notwithstanding these uncertainties, the prerequisites of the validation approach in this study are fulfilled to the extent necessary for hatch type estimation, because the intention was to place the herring within a spawning season, not to estimate their precise hatch date. Despite the possibility of underestimation of absolute age in the 0-group herring in this study, the back-calculated hatch date distributions confirm the trimodal distribution of the peak periods of spawning in winter, spring, and autumn (Figure 3).

The overlapping seasons of the autumn- and winter-spawning herring (Zijlstra, 1969; Burd and Howlett, 1974) and the gradual change in otolith microstructure from autumn hatch type through late-autumn hatch type to winter hatch type identified here appeared to result in classification of late-hatched autumn spawners as winter hatch types by visual inspection, following the guidelines currently applied. Comparing the visual inspection results in Tables 4 and 5, it is clear that most autumn-hatched herring misclassified using the classic division of hatch seasons (Table 4) were represented by fish hatched late in autumn. Therefore, visual inspection of hatch types may fail to classify individuals hatched in the periods of overlapping spawning seasons. This has potential consequences for historically splitting catches between winter and autumn hatch types for herring caught in the Skagerrak and Kattegat. However, recent efforts to separate the winter-spawning Downs component from autumn-spawning components in the North Sea (ICES, 2005) may not be affected by this problem. Downs herring otolith microstructure has been reported to be >2 μm wide at a distance of 100 μm from the nucleus (Mosegaard and Madsen, 1996), more than observed for winter-hatched herring in the present study. Consequently, it is possible that winter-hatched herring analysed in this study (i.e. the archived 0-group herring from Sub-area IIIa) did not originate from the Downs population, but from a different population of winter spawners, e.g. from the western Baltic (Bekkevold *et al*.in press).

NSS herring daily increments start at a mean increment width of 1.5 μm at the centre and gradually increase to 2.5 μm around 100 μm from the centre (Figure 2 in Husebø *et al*., 2005). This pattern is different from that observed for spring spawners in the present study, for which the mean increment width was 3 μm at 25 μm from the centre, increasing to >4 μm at 100 μm from the centre (Figure 4). The autumn spawners analysed by Husebø *et al.* (2005) also showed an increment development pattern different from that in this study.

The appearance of the otolith microstructure is much influenced by the environmental conditions, such as temperature (Folkvord *et al*., 2004) and food availability (Johannessen *et al*., 2000), experienced in the first larval phase, so caution is necessary if environmental regimes in the spawning areas change over time. The observed separation of hatch type in the present study was performed on 0-group herring sampled from year classes 2001, 2002, and 2003, and because the sampling year does have an effect on the pattern of otolith microstructure within each hatch type, a more comprehensive analysis is needed before extrapolation to other year classes can be made. This suggests the need for an annual analysis of 0-group herring otolith microstructure to update the calibration criteria for separation of hatch type by visual inspection of otolith microstructure.

In addition to the otolith microstructure patterns formed during the larval period, otolith increments formed during the juvenile growth phase may also be used to identify offspring from different spawning seasons. When the calibration sample is sufficiently large, it is possible to select a subset of segments and apply those to classify hatch types, applying a multiple linear regression model as demonstrated in the present study (Figure 5). Measurements of such defined segments provide quality checks during routine visual inspection and can aid as an additional tool to visual inspection when overgrinding of an otolith precludes application of the routine method.

The objective separation method based on median increment width of segments of otolith microstructure in the juvenile growth phase validates the use of visual inspection for hatch type separation of both juvenile and adult herring. However, it is an improvement on the visual inspection method in two ways. First, the objectivity increases the reliability of hatch type estimation of readers regardless of experience and precision level. Second, the dampening down of the inherent natural variability in otolith microstructure patterns within each hatch type by using median measurements in segments reduces misclassification errors.

As the quality and precision of hatch type estimates determined by visual inspection has depended on individual skills and

experience, the need for standardization, objective control, and statistical evaluation is obvious in improving the reliability of the output. The method developed here facilitates an objective determination of hatch type, which makes standardization and quality assurance and quality control less complicated.

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ENVIRONMENTAL CORRELATES OF POPULATION DIFFERENTIATION IN ATLANTIC HERRING

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Abstract.—The marine environment is characterized by few physical barriers, and pelagic fishes commonly show high migratory potential and low, albeit in some cases statistically significant, levels of genetic divergence in neutral genetic marker analyses. However, it is not clear whether low levels of differentiation reflect spatially separated populations experiencing gene flow or shallow population histories coupled with limited random genetic drift in large, demographically isolated populations undergoing independent evolutionary processes. Using information for nine microsatellite loci in a total of 1951 fish, we analyzed genetic differentiation among Atlantic herring from eleven spawning locations distributed along a longitudinal gradient from the North Sea to the Western Baltic. Overall genetic differentiation was low ($\theta = 0.008$) but statistically significant. The area is characterized by a dramatic shift in hydrography from the highly saline and temperature stable North Sea to the brackish Baltic Sea, where temperatures show high annual variation. We used two different methods, a novel computational geometric approach and partial Mantel correlation analysis coupled with detailed environmental information from spawning locations to show that patterns of reproductive isolation covaried with salinity differences among spawning locations, independent of their geographical distance. We show that reproductive isolation can be maintained in marine fish populations exhibiting substantial mixing during larval and adult life stages. Analyses incorporating genetic, spatial, and environmental parameters indicated that isolating mechanisms are associated with the specific salinity conditions on spawning locations.

Key words.—*Clupea harengus*, hybrid-zone, isolation by distance, local adaptation, microsatellite DNA, migration, salinity.

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Although the long held view that abundant and widely distributed marine fish are unlikely to exhibit significant genetic structure at any but the largest of geographic scales has been shown to be largely inaccurate (e.g., Ruzzante et al. 1996; Nielsen et al. 2003; 2004; O'Reilly et al. 2004; Zardoya et al. 2004), the relative roles of migratory behavior and local differences in environmentally induced selective pressures in effecting such structure remain elusive. Structure has in some cases been explained by ''isolation by distance'' (Wright 1943), which may occur when the distribution of the species is larger than the dispersal range of individuals. Isolation by distance is evident in a number of pelagic fishes and crustaceans (e.g., Palumbi 1994; Pogson et al. 2001; O'Reilly et al. 2004) but not in others (Borsa 2002; Hoarau et al. 2002; Knutsen et al. 2003; Stamatis et al. 2004). Oceanographic processes and the topography of the ocean floor have been linked to population structure in a number of species (e.g., Ruzzante et al. 1998; Norris 2000; Knutsen et al. 2004), yet few studies conducted to date have tested specifically for relationships between environmental parameters of adaptive significance and population structuring in marine migratory fish, and even fewer have examined evidence of local adaptation (Billerbeck et al. 2001; Yamahira and Conover 2002). To gauge the relative importance of various potential mechanisms for population structure in marine pelagic fishes it is useful to combine information about levels of gene flow obtained from neutral genetic markers with knowledge about migratory behavior and environmental factors that are likely to be of adaptive significance. Correlation methods have been developed to assess associations between genetic, geographic, and environmental parameters (e.g., Smouse et al. 1986; Legendre and Legendre 1998; Yang 2004). Although such methods provide a means for assessing overall associations, they do not reveal if the strength of associations are uniform over the examined spatial range. An analytical approach based on computational geometry has therefore been developed recently to address this shortcoming (Manni et al. 2004). Here, we apply such an approach to examine population structuring in Atlantic herring, *Clupea harengus* (L.), in relation to environmental heterogeneity across a small geographic, but ecologically diverse, sea area (North Sea to Western Baltic).

Atlantic herring from the North Sea–Baltic Sea transition zone provide a model system for comparing patterns of migration and gene flow and for testing hypotheses of environmentally induced barriers to gene flow in a highly migratory pelagic fish. The Atlantic herring is an iteroparous clupeoid maturing at two to three years. Herring are abundant throughout the North Sea and the Baltic, occurring in feeding and wintering shoals of mixed origin during the majority of the year (ICES 1991). Spawning takes place on rock, gravel, and/or sandy substrates at 10–50 m, and individual population

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components have traditionally been distinguished based on differences in spawning time.

The North Sea–Baltic Sea transition zone represents a major environmental gradient, as salinity changes from a full marine scale at 35‰ in the North Sea to close to zero in the inner parts of the Baltic Sea. Temperatures also differ substantially, as the North Sea confers a more stable climate than the Baltic which has shallower waters and more locally influenced temperature regimes with large annual variation. Both temperature and salinity regimes are expected to have selective impact as low temperatures impede development and survival during early larval stages (Johnston et al. 2001) and low salinity decreases fertilization and larval development success in the closely related *C. pallasi* (Griffin et al. 1998) and in other fishes from the North Sea–Baltic Sea transition area (Nissling and Westin 1997; Nissling et al. 2002).

Herring larvae are pelagic and may drift several hundred kilometers over a few months (Johannesen and Moksness 1991). Studies on tagging and morphometric character differences show that larvae spawned in autumn and winter along the British North Sea coasts drift east across the North Sea and into the Skagerrak, Kattegat, and inner Danish waters, where they feed for one to three years before returning to spawning locations in the western North Sea (Iles and Sinclair 1982). In the eastern North Sea, the Norwegian Sea, the Skagerrak, and the Baltic Sea spawning primarily takes place in spring, but with smaller autumn and winter spawning population components occurring locally (ICES 1991). The densest spawning aggregations in the area occur off the island of Rügen in the western Baltic, where spawning extends from March to May. Tagging studies have shown that Rügen spawners migrate to feeding areas in the Kattegat, Skagerrak, and the Eastern North Sea (Aro 1989). Both juvenile and adult spring spawning herring perform feeding migrations into the Skagerrak and Kattegat (Rosenberg and Palmén 1982; Johannesen and Moksness 1991), and spring spawning adults of nonlocal origin are encountered in high densities in the eastern North Sea (ICES 1991). Spawning components also occur throughout the Baltic Sea, but these and western Baltic components show no evidence of mixing (Aro 1989). However, studies also suggest that herring migratory behavior can be aberrant. Migration routes are observed to covary with changes in demography and thus appear to have some degree of social transmission (Dragesund et al. 1997).

In spite of the extensive mixing of individuals, herring population structure has been described largely from differences in morphology, growth, migration, and spawning behavior (Iles and Sinclair 1982). However, all of these traits are influenced by the physical and/or social environment that individuals experience early in life, and thus differences may be the result of phenotypic plasticity rather than of independent evolutionary trajectories. Molecular studies have provided some evidence for genetic differentiation among discrete spawning components (Shaw et al. 1999; Hauser et al. 2001; McPherson et al. 2001a; 2004; Jørstad et al. 2004; for a study employing protein markers, see also Ryman et al. 1984). In most cases levels of differentiation are estimated to be low and no study has reconciled patterns of differentiation with hypotheses about gene flow or with different selective pressures encountered locally.

The objective of this study was to use Atlantic herring sampled from spawning locations from the North Sea, the Skagerrak, and the western Baltic to (1) test the hypothesis that population structure can be maintained in a highly migratory marine fish with well described migratory patterns, and (2) to test the hypothesis that population structure correlates with environmental conditions at spawning locations. Based on highly polymorphic microsatellite DNA markers combined with detailed information about salinity and temperature regimes, we used partial Mantel tests (Legendre and Legendre 1998) and a computational geometry approach by Manni et al. (2004) to evaluate hypotheses about selectively induced barriers to gene flow. We discuss the implications of our results for predictions of local adaptation in Atlantic herring and other highly migratory marine fishes.

Materials and Methods

Sample Collection

Samples of herring (mostly) in spawning condition were collected from a total of 10 spawning locations from the eastern North Sea to the Western Baltic over a period of two years (2002 and 2003) with six of the locations sampled in both years (Table 1). One of these six locations (Rügen) was sampled repeatedly over three consecutive months in 2002 (March, April, May) and two months in 2003 (April–May). This was done to account for the temporally and presumably reproductively isolated ''spawning waves,'' which based on morphological analyses are reported to occur at this location (Rechlin 2000). No spawning herring were found in 2003 at one of the locations sampled in 2002 (Karmoy). A sample presumably representing the same Norwegian spring spawning population component, was therefore taken in 2003 at the more northerly location Møre (Fig. 1).

Individuals were aged on the basis of counts of otolith (sagitta) winter rings following the standard procedure detailed in ICES (2003). Aging was conducted by the Institute for Marine Research in Bergen, Norway, for samples from locations 6–10, and by the Danish Institute for Fisheries Research in Charlottenlund, Denmark, for samples from locations 1–5. Otolith central area microstructure was analyzed by the same laboratories to determine each individual's hatching season (spring, autumn, or winter) following a procedure described in Moksness and Fossum (1991). The percentage of ripe and running (spawning) herring was above 90% for 13 of the 20 samples (Table 1). Two samples, one from the Limfjord (2003) and one from the Kolding Fjord (2002), contained only 1% and 8% running fish, respectively. Nonrunning fish constituted a mix of fish that were about to mature and fish that had spawned recently. A sample of herring from Trinity Ledge, Nova Scotia, in the northwest Atlantic was included as an out-group for comparison (for further details about this sample, see McPherson et al. 2004).

Molecular Analyses

DNA was isolated from fin or muscle tissue using a Chelex technique (Walsh et al. 1991). For a small subset of the

TABLE 1. *Clupea harengus* samples. Locality numbers refer to Figure 1. The age distribution of each sample is shown by the numbers of individuals born in different years (1995+ indicates fish born in 1995 and earlier). Also given are mean age and percentages of spawning fish per sample. See McPherson et al. (2004) for further details on the outgroup sample from the northwest Atlantic.

Regional area	Locality	Latitude/longitude (decimal)	Sample date	Sample size	Age class							Age (years)	% ripe-and-running
					2000	1999	1998	1997	1996	1995+	Undetermined		
Western Baltic	Rügen (1)	54.23N/13.44E	22/03/02	100	—	2	16	24	37	21	—	5.69	100
			18/04/02	100	—	1	27	14	33	25	—	5.67	100
			06/05/02	100	—	1	39	9	23	28	—	4.55	100
			24/04/03	100	—	—	14	46	14	26	—	5.72	100
			06/05/03	100	—	3	31	43	9	14	—	5.12	100
Kattegat and inner Danish waters	Kolding Fjord (2)	55.49N/09.54E	12/04/02	100	—	32	36	24	2	5	1	4.10	8
			05/04/03	70	—	19	26	20	3	2	—	4.10	97
	Lillebælt (3)	55.66N/09.90E	07/04/03	100	—	1	21	18	19	40	1	5.95	50
	Kattegat (4)	55.95N/11.61E	06/05/02	44	—	11	29	2	1	1	—	3.91	74
			03/04/03	100	—	—	46	12	1	3	38	4.40	62
	Limfjord (5)	57.06N/10.06E	22/05/03	100	37	36	13	9	3	2	—	3.11	1
Skagerrak	Flatbrotten (6)	58.10N/11.33E	07/03/02	100	—	22	71	6	—	—	1	3.84	100
			19/03/03	100	—	42	30	25	—	3	—	3.99	93
	Måseskär (7)	58.34N/11.27E	19/03/02	100	—	24	67	5	1	—	3	3.82	96
			14/03/03	100	—	27	23	43	2	3	2	4.23	94
	Tjöme (8)	59.35N/10.55E	13/03/02	120	—	5	88	16	5	5	1	4.30	98
			04/03/03	120	—	2	13	62	20	9	14	5.22	100
North Sea	Karmøy (9)	59.25N/05.17E	14/03/02	100	—	—	3	3	6	80	8	7.96	100
	Møre (10)	62.78N/06.08E	17/02/03	78	—	—	—	10	5	60	3	8.31	32
Northwest Atlantic	Nova Scotia (11)	44.01N/66.31W	27/08/96	75	—	—	—	—	—	—	—	4.95	87

samples, DNA isolation was carried out using a HotSHOT technique (Truet et al. 2000). Nine tetranucleotide microsatellite loci Cha1017, Cha1020, Cha1027, Cha1202 (McPherson et al. 2001b), Cpa101, Cpa111, Cpa112, Cpa113 and Cpa114 (Olsen et al. 2002) were PCR amplified using standard reagents and annealing temperatures between 50° and 60°C (exact protocols are available on request). The loci had been chosen based on low expectations for the presence of null alleles and linkage disequilibrium (McPherson et al. 2001b; Olsen et al. 2002). An additional locus *Cpa106* (Olsen et al. 2002) was initially screened but could not be scored consistently due to presence of nonspecific peaks and was therefore omitted from the analysis. DNA fragments were visualized and genotyped using a BaseStation 51 fragment analyzer (MJ Research, Skovlunde, Denmark) in conjunction with the software Cartographer, 1.2.6 (MJ Geneworks Inc., Skovlunde, Denmark) (samples from locations 1–5), and a Pharmacia ALF-express automated sequencer in conjunction with the Fragment analyzer software (Amersham Pharmacia Biotech, Hillerød, Denmark) (samples 6–11), according to the recommendations of the manufacturers. Prior to these analyses, 10 individuals from each of four sampling locations were cross-analyzed using both methods to determine scoring consistency. No genotyping inconsistencies were observed for any of the nine loci analyzed in these 40 fish. The 40 cross-tested individuals were not included in the present study, as none was a ripe-and-running spawner, an important condition to ensure correct sampling of individual spawning components. For the present dataset, scoring consistency was continuously ascertained by double-analyzing subsets (ca. 10%) of individuals for both visualization methods. In addition, every gel included two standard individuals per locus. These standard individuals were chosen among the 40 cross-tested individuals so that their alleles together spanned the observed size ranges, which incidentally corresponded well with the size ranges observed in the full dataset.

Environmental Data

Ambient salinities and temperatures (average, maximum, and minimum) close to the sea floor (where spawning takes place and eggs are deposited) were obtained for each of the spawning locations for the month and year of sampling and for the two consecutive months. This was done to account for the time of spawning, the egg phase (7–14 days) and the larval phase (about two months). To examine the temporal stability of the hydrographic features on spawning locations, we obtained monthly average values over the years 1997–2003. Relationships between environmental parameters were examined by a series of correlation analyses using untransformed data, when Shapiro-Wilks tests indicated no deviation from normality. Environmental data were supplied by the Swedish Meteorological and Hydrological Institute, Göteborg, Sweden, by the Limfjordsovervågningen, Viborg, Denmark, and by the Institute for Marine Research, Bergen, Norway.

Statistical Analyses

Departure from Hardy Weinberg proportions (HWE) were tested for each locus and sample using GENEPOP (Raymond and Rousset 1995). The same software was used to analyze for departure from gametic phase equilibrium (linkage disequilibrium) between loci by means of ''exact tests.'' Population differentiation was estimated per sample pair and

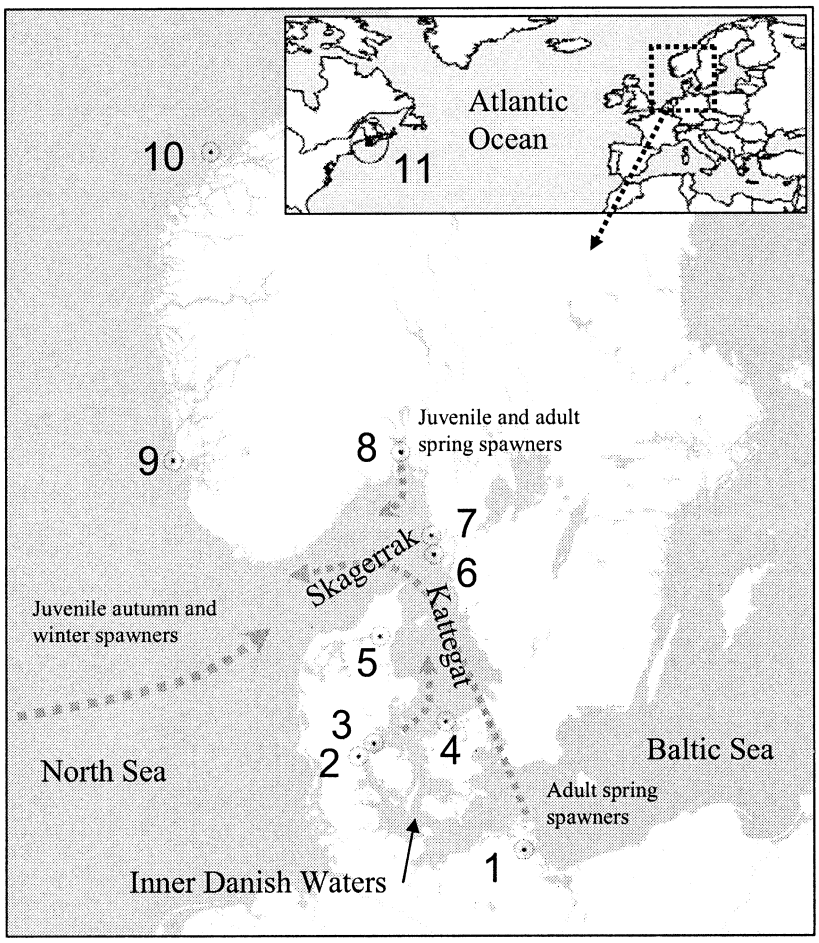

FIG. 1. Sampled *Clupea harengus* spawning locations (numbers refer to Table 1). Main migratory patterns are indicated by arrows (see text).

overall using the unbiased estimator θ (Weir and Cockerham 1984) and statistical significance was examined using permutation tests implemented in FSTAT (Goudet 2001). Temporal, within-location samples not exhibiting significant differentiation (at $\alpha = 0.05$) in these tests were pooled in subsequent analyses. FSTAT was also used to estimate allelic richness per locus and sample, using rarefaction, and to test for differences among groups of samples, using permutation tests.

In age-structured populations with overlapping generations, allele frequencies are predicted to differ among age classes due to drift, and genetic variance estimates based on varying contributions from different age classes may therefore be inflated (Jorde and Ryman 1995). To estimate potential age class effects on our results we applied hierarchical analysis of molecular variance (AMOVA, following Schneider et al. 2000) using allele frequency information from subsets of population samples, for which more than 20 multilocus genotypes were available per age class (cohort).

ViSta 5.6.3 (Young 1996) was used to perform multidimensional scaling (MDS) analysis of pairwise θ values, and to visualize genetic relationships among samples. We used the approach implemented in the software Barrier 2.2 (Manni et al. 2004) to identify shifts in genetic differentiation among spawning groups. Briefly, the method uses computational geometry and a Monmonier's maximum-difference algorithm to identify barriers to gene flow. First, a geometric map is obtained from a matrix of geographic coordinates by Voronoï tessellation, which represents the polygonal neighborhood for each sample (population) constructed so that the borders of each neighborhood are closer to the centroid (the sample coordinate) than to any other sample. A triangulation method (Delauney) is applied to connect neighboring samples into a network of triangles, and a Monmonier's algorithm is used to first determine which of the borders between neighboring populations exhibits the highest genetic differentiation. A barrier is then constructed by the algorithm continuously locating the largest genetic distance along adjacent borders and extending the barrier in a stepwise manner until reaching the outer edge of the network or another barrier. The user specifies the numbers of barriers drawn, with subsequent barriers decreasing in order of importance. We computed barriers, first by using a multi-locus θ matrix to represent genetic differentiation among samples, and second, by using θ for the nine loci separately to determine ''consensus barriers.'' The latter analysis allows for an evaluation of the robustness of barriers suggested based on the multilocus θ, by reporting

TABLE 2. *Clupea harengus* pairwise F_{ST}-values estimated by θ (below diagonal) and *P*-values (based on 1000 permutations) for pairwise significance (above diagonal). Significant F_{ST}-values following sequential Bonferroni correction ($k = 76$ tests) are indicated by asterisks (α: ***$P = 0.001$, **$P = 0.01$, *$P = 0.05$).

	Rügen March 02	Rügen Apr–May 02/03	Kolding Fjord 02	Kolding Fjord 03	Lillebælt 03	Kattegat 02/03
1. Rügen March 02		0.0061	<0.0001	0.0062	<0.0001	<0.0001
1. Rügen April–May 02/03	0.0045		<0.0001	<0.0001	<0.0001	<0.0001
2. Kolding Fjord 02	0.0097***	0.0056***		<0.0001	<0.0001	<0.0001
2. Kolding Fjord 03	0.0041	0.0047**	0.0048***		0.2085	<0.0001
3. Lillebælt 03	0.0068***	0.0025***	0.0030*	0.0016		0.0015
4. Kattegat 02/03	0.0066***	0.0029***	0.0053***	0.0033**	0.0054	
5. Limfjord 03	0.0118***	0.0032***	0.0055***	0.0064*	0.0005	0.0040***
6. Flatbrotten 02/03	0.0151***	0.0095***	0.0088***	0.0099***	0.0075***	0.0062***
7. Måseskå 02/03	0.0147***	0.0102***	0.0078***	0.0083***	0.0069***	0.0065***
8. Tjöme 02/03	0.0169***	0.0108***	0.0096***	0.0099***	0.0086***	0.0065***
9. Karmoy 02	0.0206***	0.0175***	0.0137***	0.0164***	0.0144***	0.0123***
10. Møre 03	0.0270***	0.0194***	0.0181***	0.0222***	0.0182***	0.0164***
11. Outgroup: Nova Scotia	0.0230***	0.0194***	0.0169***	0.0194***	0.0161***	0.0164***

the numbers of loci supporting the individual sections of the barriers.

Relationships among genetic, geographic, and environmental differences among spawning components were analyzed using partial Mantel tests (Legendre and Legendre 1998) implemented in the software IBD 1.5 (Bohonak 2002). Matrices of genetic (using $\theta/1 - \theta$), geographic (logarithmic shortest waterway distance, estimated using the GIS software ArcMap 8.2 supplied by ESRI (http://www.esri.com), and estimates of differences among sampling localities in each of eight environmental parameters were constructed for all pair wise population comparisons. Environmental parameters based on estimates over the years 1997 to 2003 were: (1) average salinity in spawning month, (2) minimum salinity encountered at site, (3) maximum salinity encountered at site, (4) daily variance in salinity during spawning month, (5) average temperature in spawning month, (6) minimum temperature, (7) maximum temperature, and (8) variance in temperature during spawning month. All tests were based on 10,000 randomizations and results were sequential Bonferroni corrected for multiple tests.

RESULTS

Otolith Analysis: Aging and Hatching Month

The age compositions of the sampled fish are given in Table 1. All fish in the analysis exhibited fidelity between the season they were caught as spawners and the month they had hatched (i.e., spring), as determined from the analysis of otolith structure.

Within Sample and Location Genetic Analysis

Nine microsatellite loci were analyzed in 1951 fish. Scoring success overall was high as 97% of all individuals were genotyped at all nine loci (see Appendix available online at http://dx.doi.org/10.1554/05-183.1.s1). Repeated analyses of subsets of individuals for each of the two sequencing methods demonstrated high genotyping consistency across methods, because across loci and samples 98.15% of the alleles were scored consistently. Following correction for 180 multiple tests, three Hardy Weinberg proportions tests in three different loci indicated significant deviations (see Appendix available online). If a less conservative correction factor of $k = 20$ was applied, significance was indicated for a total of eight deviations. Because the deviations were distributed across loci and samples and since none was associated with a large F_{is}, we do not attribute them to null alleles. No significant effects of gametic phase disequilibrium were found across loci and samples. Of the seven within-location temporal comparisons, only the samples from Kolding Fjord exhibited significant differentiation (θ estimated at 0.0048 [95% CI = 0.0023–0.0080]). Within the Rügen location, only the March 2002 sample exhibited low but significant differentiation from any of the other Rügen samples (θ ranging between 0.003 and 0.008 in four pairwise comparisons). This sample was therefore entered separately into subsequent analyses, whereas samples from April and May 2002 and 2003 were pooled.

Spatial Population Differentiation

Estimates of pairwise population differentiation generated for pooled temporal samples ranged between −0.0002 and 0.0270 (Table 2), and the overall θ was estimated at 0.008 (95% CI = 0.004–0.013, $P < 0.001$). The Nova Scotia outgroup sample was significantly differentiated from all samples, except the two geographically closest North Sea samples (Table 2). An AMOVA incorporating data for 1997, 1998, and 1999 age classes nested within the Rügen, Kolding Fjord, and Tjöme sampling locations did not indicate genetic differences among age classes within locations (−0.21% variance explained, $P = 0.998$), whereas a highly significant proportion of the genetic variation was explained by differentiation among spawning locations (0.93% variance explained, $P < 0.001$). Allelic richness varied among samples for all loci (Appendix 1) and (averaged over loci) decreased from the North Sea and Skagerrak to the Baltic (North Sea [two samples] $R_s = 14.93$; Skagerrak [three samples] $R_s = 15.31$, Kattegat and inner Danish waters [five samples] $R_s = 13.98$; Western Baltic Sea [two samples] $R_s = 13.44$; $P = 0.002$).

Samples grouped along the first dimension axis in the MDS analysis in general correspondence with their longitudinal

TABLE 2. Extended.

Limfjord 03	Flatbrotten 02/03	Måseskär 02/03	Tjöme 02/03	Karmoy 02	Møre 03	Nova Scotia
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.3520	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0.0073***		0.0576	0.1306	0.1097	<0.0001	<0.0001
0.0083***	0.0002		0.2180	0.0257	<0.0001	<0.0001
0.0090***	0.0002	−0.0002		<0.0001	<0.0001	<0.0001
0.0158***	0.0005	0.0015	0.0022***		0.4866	0.0203
0.0170***	0.0033***	0.0046***	0.0035***	0.0000		0.0049
0.0173***	0.0033***	0.0033**	0.0036***	0.0017	0.0037	

position from the North Sea to the Baltic, except for the Northwest Atlantic ''outgroup'' sample which grouped between North Sea samples (Fig. 2A). The first axis explained 68% of the variation. The second axis, explaining 9% of the variation, indicated separation between Skagerrak and North Sea samples. Subsequent dimensions each explained less than 8% of the variance and were not considered further. In the BARRIER analysis the first reproductive barrier, which was supported by five to eight loci over different sections of the barrier, separated North Sea and Skagerrak populations from all other samples (Fig. 2B). The second barrier, supported by five and six loci, separated the Rügen March sample from all other samples. The third and fourth barriers, both supported by four to five loci, separated respectively, the Kolding Fjord 2003 sample from all other samples, and the Kattegat and Rügen samples from all other samples. Subsequent barriers were supported by only two to four loci and not considered further.

Hydrographic Environment and IBD

Across the ten sampling locations, strong positive correlations were found between average salinity in the first three months following spawning (all $r > 0.94$), indicating that hydrographic differences among locations remained constant during the egg- and first-larval stages. Lesser but similar trends were found for temperature (all $r > 0.56$). Positive correlations were also observed for both salinity and temperature in the year and month of sampling compared to averages over the years 1997-2003 ($r_{\text{salinity}} > 0.97$; $r_{\text{temperature}} > 0.85$), indicating that conditions in the years represented in our samples were similar to those encountered over a six to seven year period and presumably longer. Figure 3 shows trends in salinities and temperatures in the month of sampling for the ten locations. Differences among sampling locations in average salinity, maximum and minimum salinity were correlated with each other (all $r > 0.94$). No other correlations were detected between any of the other environmental parameters examined.

Mantel tests indicated significant correlations between genetic differentiation and average and minimum salinity at spawning locations, which persisted when controlling (partial Mantel test) for geographic distance, whereas the correlation between genetic and geographic distances was nonsignificant or marginally significant when the environmental components (monthly average, monthly maximum and monthly minimum salinity) were controlled for (Table 3). Tests including maximum salinity, salinity variance, or any of the temperature parameters gave no evidence of covariance with genetic differentiation (Tables 3, 4).

DISCUSSION

Differentiation among Spawning Locations

We identified temporally stable differentiation among spawning locations along an environmental gradient despite the fact that individuals migrate freely across it. Due to annual variation in recruitment success our samples varied in age composition between the two sampling years (Table 1). Unequal sampling of potentially differentiated age classes may lead to erroneous conclusions about spatial versus temporal allele frequency variance (Jorde and Ryman 1995), but the AMOVA indicated that no detectable variability was found among age classes sampled within locations. Estimates of population differentiation are commonly almost an order of magnitude lower in marine fishes compared to estimates in freshwater fishes and terrestrial organisms. Summarizing estimates from a range of marine fishes Ward et al. (1994), for example, reported a median F_{ST} of 2%. In comparison, the overall F_{ST} estimated for our population samples was less than 1%, whereas between-individual sample estimates came up to 2.7% (Table 2). Proper interpretation of such low levels of differentiation requires consideration of the negative relationship between maximal attainable F_{ST} and level of polymorphism of the genetic markers used (very high in the loci employed here; c.f. Appendix 1)(Hedrick 1999). Regardless, it is pertinent to question the biological significance of low F_{ST} values. One way of doing so is to examine whether observed patterns are temporally stable (Waples 1998). We therefore consider the fact that the observed patterns of genetic differentiation were stable across two years of sampling evidence for their biological significance. Moreover, the samples showed a clear grouping along a west-east axis corre-

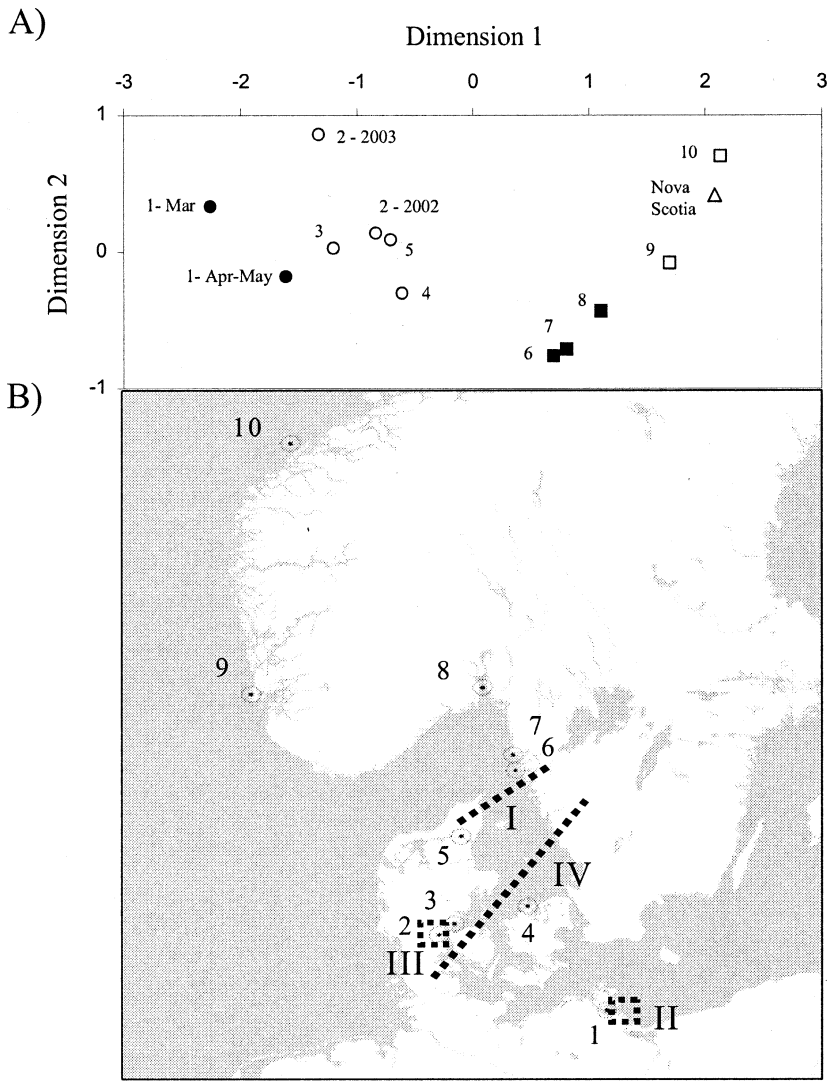

FIG. 2. (A) MDS plot of samples from the Western Baltic (closed circles), Kattegat and inner Danish waters (open circles), Skagerrak (closed squares), North Sea and Norwegian Sea (open squares), and the Northwest Atlantic outgroup (triangle). Locality numbers refer to Table 1. Respectively, 68% and 9% of the variance was explained by the first and second axes (stress = 0.16). (B) Major shifts in gene flow suggested by BARRIER, with the order of importance given in roman numerals. The second and third barriers separate, respectively, the Rügen March sample from all other samples and the Kolding Fjord 2003 sample from all other samples.

sponding with the North Sea–Baltic Sea transition zone (Fig 2A). The sets of genetic markers applied in our study were associated with high power for detection of even very small allele frequency differences among samples (Ryman et al., pers comm). Thus, our study represents the first demonstration of a well-defined spatial relationship for the genetic structure in a clupeoid fish.

The levels of genetic differentiation detected in our study were higher than those reported for *C. harengus* populations across comparable geographical ranges in the northwestern Atlantic, where no relationship between geographic and genetic differentiation is evident (McPherson et al. 2004). A microsatellite study of autumn and winter spawning *C. harengus* populations spanning a >1000 km north-south transect in the western North Sea reported very low, albeit statistically significant differentiation (F_{ST} = 0.1%, $P < 0.001$) and lack of distinct geographical structure in this area (Mariani et al., in press). We found that northwestern Atlantic (Nova Scotia) and northeastern Atlantic (North Sea) spawning components did not differ genetically (Table 2). Lack of differentiation of neutral genetic markers across scales of several thousands of kilometers may reflect recent colonization histories coupled with large populations experiencing low levels of genetic drift (Grant and Bowen 1998) and need not invoke high gene flow per se. The large contrast detected in levels of differentiation among samples within the Atlantic and those between the Atlantic and the Baltic show that levels of gene flow can be expected to be highly variable over the distribution of the species.

Failing to follow a sound sampling scheme is likely to lead to erroneous conclusions about population structure in highly migratory organisms (Carvalho and Hauser 1997). Our samples included high proportions of ripe and running fish and hence satisfy the requirements for sampling of reproductively

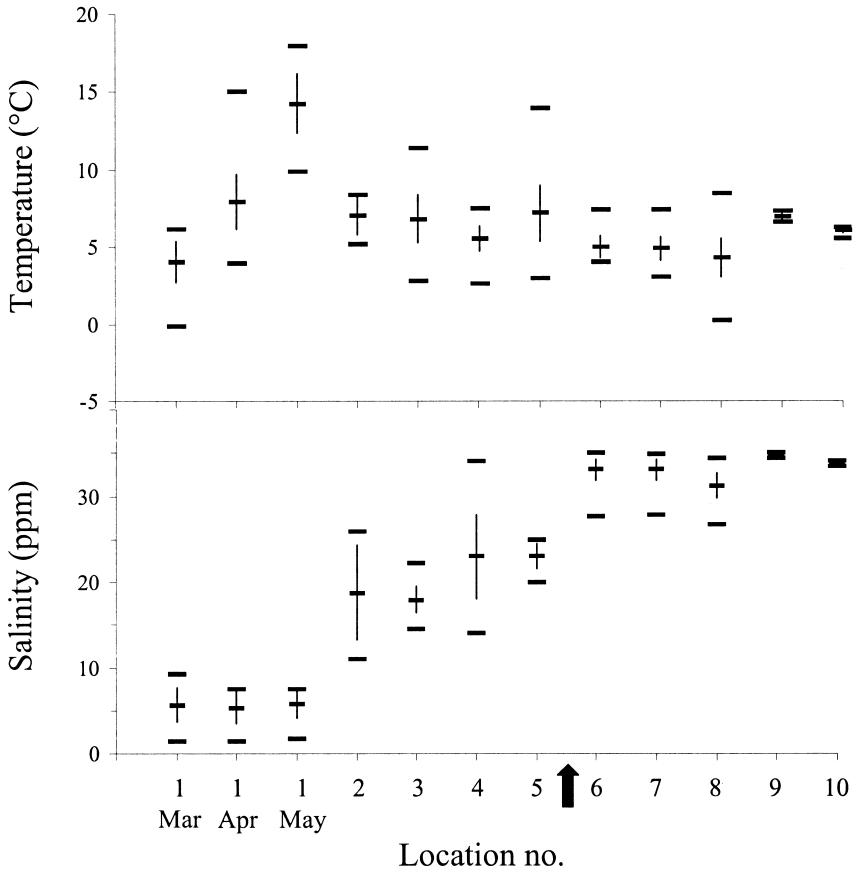

FIG. 3. Temperature (upper diagram) and salinity (lower diagram) on spawning locations in the month of sampling. Values are averages over the years 1997–2003, error bars indicate standard deviations estimated over days across years, and vertical bars indicate local maxima and minima. Location numbers refer to Figure 1. Values for location 1 are presented for each of the three sampling months (see text). The arrow indicates where the computational geometric analysis indicated the largest discontinuity in gene flow among spawning locations.

TABLE 3. Mantel tests (regular and partial) for matrix correlations incorporating pairwise differences in genetic, geographic, and each of four salinity parameters. Significant (at $\alpha = 0.05$) *P*-values following correction for four sequential tests are indicated with asterisks. Test results for genetic versus geographic distances are similar across the four analyses and are only given once.

Salinity parameter	Average salinity			Minimum salinity			Maximum salinity			Salinity variance		
	Z	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2
Genetic vs. geographic distance	1.507	<0.001*	0.145									
Partial, controlling for environmental parameter		0.306	0.005		0.883	0.027		0.009*	0.099		0.002*	0.122
Genetic vs. environmental difference	9.034	<0.001*	0.426	10.401	<0.001*	0.598	7.308	0.014	0.129	1.374	0.033	0.078
Partial, controlling for geographic distance		<0.001*	0.332		<0.001*	0.542		0.039	0.082		0.063	0.052

TABLE 4. Mantel tests (regular and partial) for matrix correlations incorporating pairwise differences in genetic, geographic, and each of four temperature parameters. Significant (at $\alpha = 0.05$) *P*-values following correction for four sequential tests are indicated with asterisks. Results for genetic versus geographic distances are similar across tests and are only given once.

Temperature parameter	Mean temperature			Minimum temperature			Maximum temperature			Temperature variance		
	Z	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2	*Z*	*P*	r^2
Genetic vs. geographic distance	1.507	<0.001*	0.145									
Partial, controlling for environmental parameter		<0.001*	0.156		0.002*	0.127		<0.001*	0.149		<0.001*	0.189
Genetic vs. environmental difference	1.138	0.156	0.043	1.477	0.082	0.042	1.862	0.239	0.012	1.613	0.954	0.068
Partial, controlling for geographic distance		0.126	0.056		0.148	0.021		0.203	0.016		0.989	0.116

coherent components. At the one locality where temporal allele frequency stability was rejected (Kolding Fjord 2002 vs. 2003) the sample from 2002 contained only 8% ripe individuals and may not have represented the local spawning component adequately. This sample showed closest similarity with samples collected at locations outside the fjord (Fig. 2A), suggesting that a proportion of the sampled fish originated from neighboring locations. The Limfjord sample also contained few ripe and running fish, but the single temporal sample prevented determination of whether fish were local or comprised of migrants originating elsewhere. The main access to the Limfjord is via the eastern mouth of the fjord, and although Limfjord herring are assumed to perform feeding migrations into the Kattegat, the relative isolation of the fjord nonetheless suggests that individuals present around the time of spawning would be of local origin.

We found some indication for reproductive isolation between short-term temporally differentiated ''spawning waves'' at the Rügen location. The early (March) sample was significantly differentiated from later samples (Table 2), but our samples did not allow tests of annual stability. Within-year samples showed slightly differing age class distributions because older fish tended to spawn earlier than younger fish (Table 1), and tests for differentiation among short-term temporal samples using subsets of individuals belonging to the same age class revealed no evidence for structure (results not shown). Although we could not determine the biological significance of the short-term genetic differentiation at this location, our results emphasize the importance of sampling scheme (i.e., equal cohort representation and assuring that samples represent spawning individuals) in analyses of temporal stability. Failure to recognize local substructure would, for example, be critical to attempts at generating estimates of effective population size and could lead to severe underestimation of this evolutionarily important parameter.

Environmental Correlates of Population Structure

Applying Mantel tests we demonstrated a signal of isolation by distance, but also that both minimum and average salinity exhibited high correlations with genetic differentiation. The latter trends persisted when effects of geographic distance were controlled for using partial Mantel analysis, whereas conversely the trend for isolation by distance disappeared when these environmental parameters were held constant (Table 3). Caution should be taken when relationships between geographic, population genetic, and environmental parameters are examined by means of partial Mantel tests (Raufaste and Rousset 2001). Multicollinearity between independent variables, in our case geographic distance and environmental parameters, is likely to lower power through inflated type-I errors in tests of the partial correlation between the environmental parameter and the dependent variable, in this case genetic differentiation (Castellano and Balletto 2002). Nonetheless, the highly significant correlation between salinity and genetic differentiation when controlling for geographic distance showed that salinity parameters and/or associated factors were correlated with gene flow among spawning locations, and suggested that salinity differences rather than distance per se affected levels of reproductive isolation among spawning components. In the North Sea–Baltic Sea transition zone salinity, or factors correlated with salinity, may act as cues for homing to spawning grounds. Factors associated with salinity parameters and the timing of juvenile and adult migratory behavior may also act to produce a strong signal of reproductive isolation being associated with salinity. We stress that the analysis does not demonstrate local adaptation to a specific salinity regime (see below) but that our approach offers a means of comparing genetic and various environmental parameters to evaluate the relative magnitudes of their covariances.

Our analyses allowed us to compare environmental patterns with the BARRIER computational geometric approach. The BARRIER analysis indicated that the largest change in gene flow (i.e., the largest reproductive isolation) occurred between spawning locations in the Skagerrak and Kattegat (Fig. 2B). Although the statistical significance of this result could not be determined directly, the consensus analysis showed that the barrier was supported by multiple independent loci. The delimitation between these areas corresponds with a major transition in salinity parameters and a general trend for a more stable environment northwest of the barrier, as opposed to more variable local conditions southeast of the suggested barrier (Fig. 3). The second to fourth identified barriers were supported by fewer loci and only partially corresponded with environmental changes. The low salinity in the Baltic relative to the inner Danish waters was, for ex-

ample, only partly associated with a signal of reproductive isolation (compare Fig. 2B with Fig. 3).

The computational geometric approach BARRIER enables visualization of hierarchical patterns of differentiation, irrespective of actual magnitudes of differentiation. Interpretation of the biological significance of barriers should therefore be treated with caution and ideally be combined with other types of evidence for isolating mechanisms, such as behavioral and environmental factors. A caveat when applying the approach to populations following an isolation-by-distance model occurs when the sampled locations are not equally spaced geographically (Manni et al. 2004). Here, the algorithm will return a signal of a reproductive barrier between the geographically, and hence genetically most distant neighboring populations. In such cases, it is not possible to determine whether the constructed barrier reflects a true barrier to gene flow or merely that information for intermediate populations was not included in the analysis. We identified the largest increase in levels of reproductive isolation in regions that were relatively well represented by samples, indicating that the identified barrier is unlikely to reflect mere sample coverage. Moreover, the area in which the highest level of population differentiation was observed encompassed an ecologically meaningful gradient that is likely to impact growth and recruitment.

The largest reproductive barrier was estimated between groups of samples scored using different genotype visualization techniques and inconsistent scoring between approaches could potentially have affected this analysis. Our quality control measures and the high resulting consistency between techniques, together with the combined evidence from the spatially explicit distribution of samples shown in Figure 2A and the fact that the barrier was supported by multiple individual loci strongly suggest that the result is unlikely to have been caused by technical artifacts.

The barriers to gene flow detected between spawning components could presumably result from distinct hydrographic circulation systems that retain larvae and/or adults within local areas, as has been suggested for herring (Iles and Sinclair 1982) and other species including cod, *Gadus morhua* (Ruzzante et al. 1998); sea bass, *Dicentrarchus labrax* (Bahri-Sfar et al. 2000); and marine invertebrates with pelagic larvae (Wares et al. 2001). However, in the herring populations studied here, juveniles and adults are not retained within natal areas. The North Sea hydrographic circulation transports autumn hatched larvae from the east coast of the United Kingdom to juvenile nursery areas in the Skagerrak and the Kattegat (Rosenberg and Palmén 1982, Fig. 1), and tagging studies on spring spawning western Baltic herring from the Rügen area indicate feeding migrations to the same areas (Aro 1989). The proportions of the two major groups in the area of mixing have previously been based on analyses of differences in growth and maturity combined with metrics like vertebrae numbers (ICES 1991). Otolith microstructure analysis has been used to identify the hatching season of mixed individuals and has indicated the existence of several subcomponents among spring spawners feeding in the Skagerrak-Kattegat area (Mosegaard and Madsen 1996; Mosegaard et al. 2001), and a genetic study corroborates this (Bekkevold et al., unpubl. ms.). These observations hence refute that the population structure reported here is caused solely by physical isolation between population components, and instead invoke a role for active homing and possibly locally differentiated selection pressures.

The Potential for Local Adaptation in Herring

A number of studies have sought to use divergence in ecologically relevant habitat factors as a surrogate of divergent selection to examine associations with gene flow (e.g., Smith et al. 1997; Reusch et al. 2001; Ogden and Thorpe 2002). We identified correlations between population differentiation and environmental (salinity) parameters associated with the spawning, egg, and early larval phase that were of greater magnitude than correlations between geographical and genetic differentiation (Table 3). Whereas such observations do not demonstrate that genetic differentiation is maintained by selection, salinity parameters are expected to exert strong selective pressures in marine organisms. Fertilization and larval developmental success are, for example, reduced at both low and high salinity levels in Pacific herring, *C. pallasi*, and this species' optimal salinity range was found to be higher than that of Baltic herring (Griffin et al. 1998). Baltic herring spawn in coastal low salinity habitats where larval retention is high due to limited large-scale hydrographical activity (Lehmann et al. 2002) and therefore developing larvae have high probability of experiencing a predictable environment. Across the North Sea–Baltic Sea transition zone spatially variable but locally predictable environmental conditions suggest that herring from different spawning locations experience stabilizing selection for different salinity tolerance optima. Locally differentiated selective pressures would consequently lead to selection against dispersal between spawning locations of differing salinity conditions, and may provide an adaptive explanation for the homing behavior reported in herring tagging studies (reviewed by McQuinn 1997). Based on estimates of dispersal between spawning locations, levels of gene flow have previously been considered to be substantial and to preclude local adaptation in herring (McQuinn 1997). However, gene flow constrains, but need not preclude, adaptive evolution (e.g., King and Lawson 1995; Calsbeek and Smith 2003; Saint-Laurent et al. 2003; Hendry et al. 2004). Moreover, rates of dispersal between populations cannot be directly translated into realized gene flow, because the reproductive success of dispersers and their offspring is influenced by local selective pressures. The relative fitness returns associated with dispersing versus philopatric behavior are at present unknown in herring, as in most other marine fishes. The present study suggests that salinity conditions on spawning locations affect the fitness associated with different dispersal behaviors. Common garden experiments, such as applied by Billerbeck et al. (2001), could be used to evaluate such effects.

Evidence for a Multispecies North Sea–Baltic Sea Hybrid Zone?

Clines in neutral genetic markers are often associated with ecotone shifts and hybrid zone boundaries (Barton and Hewitt 1985). Our study complements genetic studies in other fishes from the North Sea–Baltic Sea transition zone. Two recent

studies identified genetic clines in the western Baltic in Atlantic cod (Nielsen et al. 2003) and turbot, *Scopthalamus maximus* (Nielsen et al. 2004). In both species, relatively low differentiation was indicated among samples within both the North Sea and the Baltic Sea, and the authors suggested that genetic patterns reflect the presence of a hybrid (interaction) zone between ''pure'' Baltic and North Sea populations. We sampled single spawning components from the Baltic and the North Sea only, and thus were not able to evaluate levels of differentiation within each of these areas. However, two recent microsatellite studies reported, respectively, population structure in Baltic Sea herring (Jørgensen et al. 2005), and limited structure in North Sea autumn and winter spawning components (Mariani et al. in press). Presence of a hybrid zone can be evaluated by examination of linkage disequilibria through the suspected zone of interaction (e.g., Barton 2000). However, the relatively low levels of differentiation detected in most marine fishes including herring using neutral genetic markers requires analysis of large numbers of loci to obtain sufficient statistical power for demonstrating linkage, and was not attempted here. We evaluated spatial differences in genetic variability by estimating allelic richness and found that variability decreased from the North Sea to the Baltic. A similar trend was reported in Nielsen et al.'s studies of cod and turbot and in the aquatic plant *Zostera marina* from the same area (Olsen et al. 2004). Such patterns correspond with founder events following colonization from the North Sea via the Skagerrak when the Baltic Sea was created following glacial retreat about nine thousand years ago. Based on our data it is not possible to determine whether the observed population structure reflects the existence of a hybrid zone where selection acts against hybrids (endogenous selection), or of multiple reproductively isolated spawning components, each exhibiting maximal fitness in native environments (exogenous selection). Whether the former or latter scenario yields better explanatory power has implications to predictions about population fitness curves through the area, and about the temporal stability of the genetic cline (Dasmahapatra et al. 2002). However, such congruity in the geographic positioning of the largest reproductive barrier in herring and the distribution of cline centers reported for both cod and turbot (Nielsen et al. 2003; 2004) (to within a few hundred kilometers) further suggests that spatial genetic variance in these three species is related to selective differences along a common ecological gradient, rather than a mere result of secondary contact between allopatrically differentiated North Sea and Baltic population components, as has been suggested for *Macoma balthica* for example (Luttikhuizen et al. 2003).

Conclusions

We have shown that genetic structure can be maintained in marine fish populations exhibiting substantial mixing during larval and adult life stages. Analyses incorporating genetic, spatial, and environmental parameters indicated that isolating mechanisms are associated with the specific salinity conditions on spawning locations. Our results do not imply a role for linkage between microsatellite DNA loci and traits under selection, but that populations experiencing dissimilar salinity conditions on spawning locations follow different evolutionary trajectories. This shows that the North Sea–Baltic Sea transition zone offers an insightful opportunity for studying local adaptation in ''classical'' marine fishes with continuous distributions, such as herring, cod, and turbot. Little is yet known about selective patterns in species inhabiting the open sea, although it is evident that geographic separation and dispersal potential are poor predictors of the spatial scale of the potential for local adaptation in marine systems. Our approach of combining results from partial Mantel tests with the computational geometric approach offers a promising means of evaluating relationships between barriers to gene flow and environmental variance across marine fishes and ecosystems, as it can be applied both within and across species.

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Chapter 3: Characterising the mixture of herring in the Study area

The herring soup.

Paper 3:

Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring

Daniel E. Ruzzante, Stefano Mariani, Dorte Bekkevold, Carl André, Henrik Mosegaard, Lotte A. W. Clausen, Thomas G. Dahlgren, William F. Hutchinson, Emma M. C. Hatfield, Else Torstensen, Jennifer Brigham, E. John Simmonds, Linda Laikre, Lena C. Larsson, Rene´ J. M. Stet, Nils Ryman and Gary R. Carvalho

The existence of biologically differentiated populations has been credited with a major role in conferring sustainability and in buffering overall productivity of anadromous fish population complexes where evidence for spatial structure is uncontroversial. Here, we describe evidence of correlated genetic and life history (spawning season linked to spawning location) differentiation in an abundant and highly migratory pelagic fish, Atlantic herring, Clupea harengus, in the North Sea (NS) and adjacent areas. The existence of genetically and phenotypically diverse stocks in this region despite intense seasonal mixing strongly implicates natal homing in this species. Based on information from genetic markers and otolith morphology, we estimate the proportional contribution by NS, Skagerrak (SKG) and Kattegat and western Baltic (WBS) fish to mixed aggregations targeted by the NS fishery. We use these estimates to identify spatial and temporal differences in life history (migratory behaviour) and habitat use among genetically differentiated migratory populations that mix seasonally. Our study suggests the existence of more complex patterns of intraspecific diversity than was previously recognized. Sustainability may be compromised if such complex patterns are reduced through generalized management (e.g. area closures) that overlooks population differences in spatial use throughout the life cycle.

Paper 5:

Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area

Dorte Bekkevold, Lotte A. W. Clausen, Stefano Mariani, Carl André, Emma M. C. Hatfield, Else Torstensen, Nils Ryman, Gary R. Carvalho, Daniel E. Ruzzante

Determining spatio-temporal distributions of fish populations is of interest to marine ecology, in general, and to fisheries science in particular. Genetic mixed-stock analysis is routinely applied in several anadromous fishes for determining migratory routes and timing but has rarely been used for marine fishes, for which population differentiation is commonly weak and the method presumably less powerful. We used microsatellite information for Northeast Atlantic herring Clupea harengus L. populations and mixed stocks to address 2 questions. We used simulated mixture samples and 3 different statistical approaches to determine whether mixed stock composition could be determined with accuracy. Simulations showed that the applied approaches and mixture samples of 100 individuals enabled detailed composition analyses on a regional level, with resolution for tracing the ecologically dominant Rügen (Greifswalder Bodden) herring population. We then estimated spatio-temporal variation in herring migratory behaviour in the Skagerrak from 17 mixed samples collected over 2 seasons and 2 yr, and identified hitherto undescribed differences in distributions among populations that feed and winter in the area.

Paper 6:

Effect of spatial differences in growth on distribution of seasonally co-occurring herring stocks

Lotte A. W. Clausen, Karl-Johan Stæhr, Anna Rindorf and Henrik Mosegaard

The mechanisms most likely to determine the distribution of the two major herring stocks in their common early summer feeding ground in the Eastern North Sea, Skagerrak and Kattegat were investigated through analysis of acoustic survey data from six consecutive years. No change was detected in biomass of North Sea Autumn Spawning herring (NSAS) over time whereas the biomass of Western Baltic Spring Spawning herring (WBSS) severely declined. Analyses of centre of gravity by stock showed no change in NSAS distribution, whereas the WBSS changed to a more western distribution over time. Contrary to previous perception of the juvenile migration, NSAS were found to leave the study area already at the age between 1 and 2 and WBSS 1 year olds were encountered in the Skagerrak. The estimated parameters of von Bertalanffy growth equations showed marked differences between areas with fish in the eastern part of the area having the lowest size at age at all ages. Further, their growth conditions appeared to deteriorate progressively over the period studied. Both NSAS and WBSS showed the highest condition in the North Sea and Skagerrak while condition was substantially lower in Kattegat. The westward movement of spring spawners over time suggests that growth rate and possibly density of conspecifics influences the migration pattern and distribution of herring in the area. In contrast, there was no evidence to suggest that distribution was constant over time within stocks or that distribution reflected size dependent limitations on migration distance.

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Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring

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The existence of biologically differentiated populations has been credited with a major role in conferring sustainability and in buffering overall productivity of anadromous fish population complexes where evidence for spatial structure is uncontroversial. Here, we describe evidence of correlated genetic and life history (spawning season linked to spawning location) differentiation in an abundant and highly migratory pelagic fish, Atlantic herring, *Clupea harengus*, in the North Sea (NS) and adjacent areas. The existence of genetically and phenotypically diverse stocks in this region despite intense seasonal mixing strongly implicates natal homing in this species. Based on information from genetic markers and otolith morphology, we estimate the proportional contribution by NS, Skagerrak (SKG) and Kattegat and western Baltic (WBS) fish to mixed aggregations targeted by the NS fishery. We use these estimates to identify spatial and temporal differences in life history (migratory behaviour) and habitat use among genetically differentiated migratory populations that mix seasonally. Our study suggests the existence of more complex patterns of intraspecific diversity than was previously recognized. Sustainability may be compromised if such complex patterns are reduced through generalized management (e.g. area closures) that overlooks population differences in spatial use throughout the life cycle.

Keywords: homing; genetic mixture analysis; diversity conservation; pelagic fisheries

1. INTRODUCTION

Marine conservation initiatives or fisheries management regimes that disregard or misidentify patterns of genetic and life-history differences among migratory populations that mix seasonally (i.e. intraspecific component of biocomplexity), have the potential to result in the erosion of genetic resources. This problem is especially acute for marine fish population complexes with diverse and potentially locally adapted migratory components that overlap spatially and seasonally. In such complex systems, the more easily exploited or the less-productive components will also be the most easily eliminated with the consequent loss of diversity and adaptive potential (Iles & Sinclair 1982; Policansky & Magnuson 1998). Although it is widely accepted that the loss of genetic diversity is likely to have profound negative effects on recruitment potential and population recovery (Ryman *et al.* 1995), the significance, and in fact the very existence of such biologically relevant (i.e. spatially and temporally explicit) diversity for highly abundant and widely distributed migratory marine fish, has traditionally been controversial (McQuinn 1997). Given the documented historical loss of species diversity in coastal ecosystems through extinctions caused by overfishing (Jackson *et al.* 2001), and the worldwide depletion of fish populations and communities (Pauly *et al.* 1998; Myers & Worm 2005) there is an urgent

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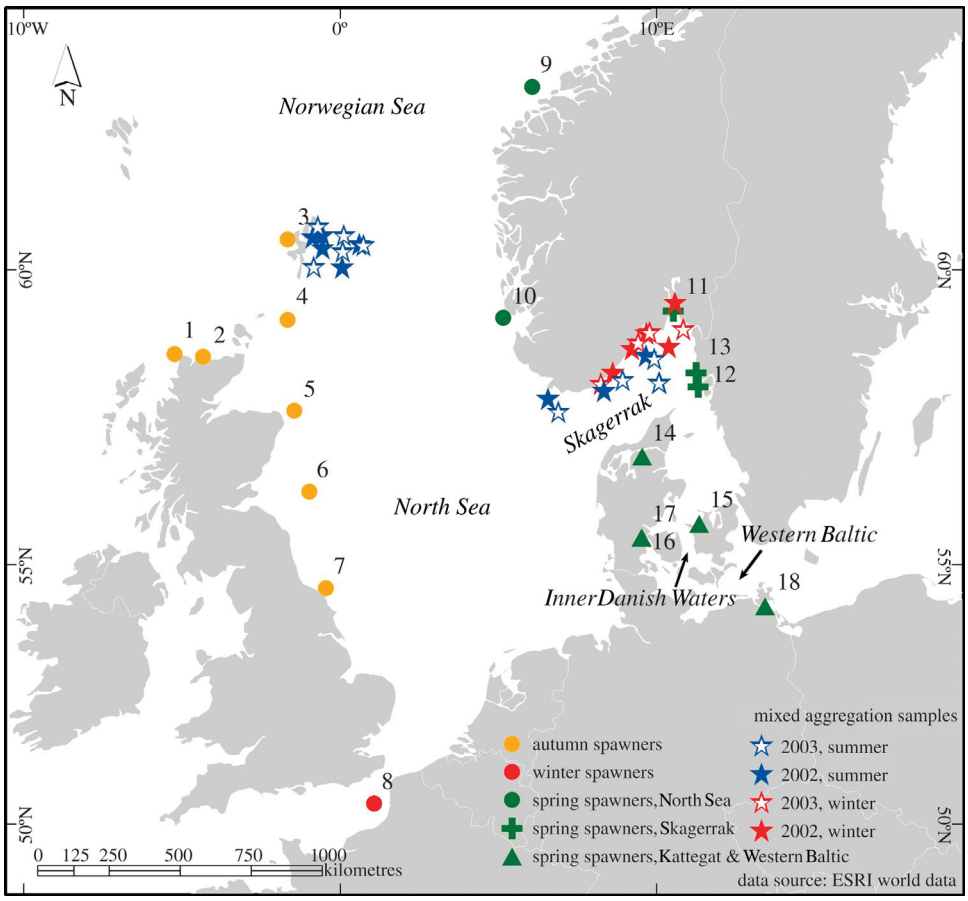

Figure 1. Sampling locations for herring in spawning condition and for herring in mixed feeding or overwintering aggregations. Spawning locations are numbered as in electronic supplementary material, table A1). For the MSA, locations 1–10, 11–13, and 14–18 were pooled into: region *1* ($n=1332$) comprising autumn (filled circle in orange), spring (filled circle in green) and winter (filled circle in red) spawners in the North Sea; region *2* ($n=635$), comprising spring spawners from the Skagerrak (plus), and region *3* ($n=1010$), comprising spring spawners (filled triangle in green) from the Kattegat and Western Baltic. The locations for the mixed aggregations are indicated by stars, solid stars (2002), open stars (2003): blue, summer feeding aggregations; red, overwintering aggregations, respectively. See electronic supplementary material for details.

need to delineate extant patterns of within-species genetic diversity and to use such knowledge to quantify the relative contributions of population components to mixed fisheries. This need applies to marine fishes in general, but particularly to species such as herring, which in the past have exhibited large and persistent local spawning aggregations supporting human communities entirely dependent on their fishery (Alheit & Hagen 1997), and for which local extinction/recolonization events are well documented for at least the last century (Corten 2002).

Our research was designed to examine spatial structure in herring (*Clupea harengus harengus* L., Clupeidae, Teleostei) in the North Sea (NS) and adjacent areas and to use such knowledge to estimate the contribution by different herring assemblages to feeding aggregations targeted by the fisheries.

2. MATERIAL AND METHODS

(a) *Sample collection*

Temporally replicated samples of spawning herring were obtained in 2002 and 2003 (one sample collected in November 2001) from 18 spawning aggregations spanning the distributional range of herring in the NS and adjacent areas (global $n=2997$; figure 1, electronic supplementary material, table A1). Herring samples were also obtained from summer feeding aggregations in the northern North Sea (NNS) and Skagerrak (SKG) (July 2002 and 2003, NNS $n=492$ and 472, SKG $n=600$ and 400 respectively, figure 1; electronic supplementary material, table A2) and from overwintering aggregations in the SKG (December 2002 and 2003, $n=500$ and 400 respectively, figure 1, electronic supplementary material, table A2). All samples were analysed genetically with a suite of 9 microsatellite DNA loci (electronic supplementary material, table A3), and phenotypically for a number of otolith winter rings (proxy for age; ICES 2003) and otolith microstructure (proxy for season individual was hatched; Moskness & Fossum 1991).

(b) *Molecular analysis*

A total of $n=5841$ herring were screened with a suite of 9 microsatellite DNA loci (details available in electronic supplementary material). DNA extraction and PCR amplification procedures as well as information on population structure based on the collections of spawning herring are reported in Mariani *et al.* (2005) for the NS and in Bekkevold *et al.* (2005) for the SKG/Kattegat/WBS. The genetic composition of the feeding aggregations (global $n=2864$, details available in electronic supplementary material) is reported in the present paper.

(c) *Phenotypic analysis*

Otolith (sagitta) winter rings were counted as a proxy for age following standard procedures (ICES 2003). Ageing was

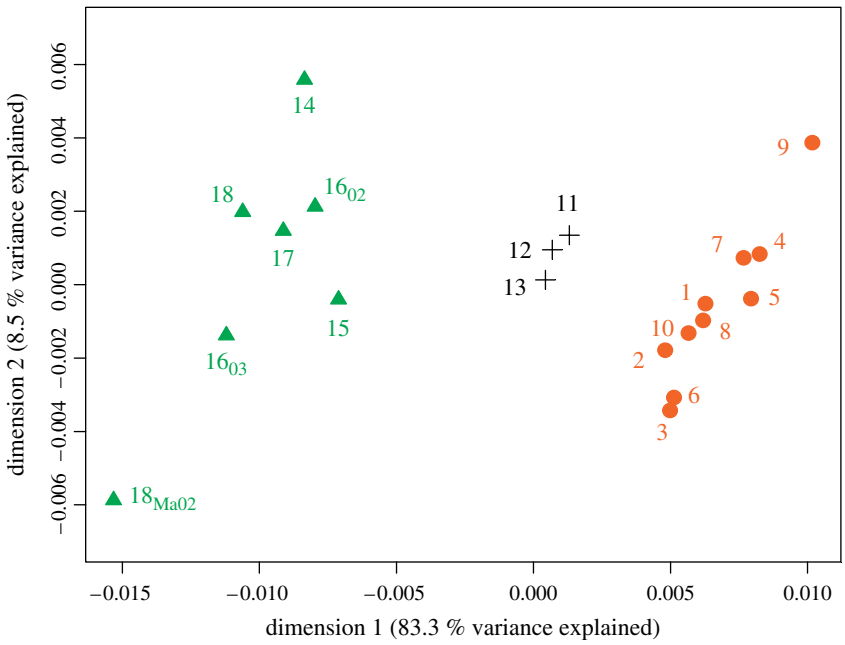

Figure 2. Multi-dimensional scaling plot of pairwise F_{ST} estimates between herring spawning collections. Temporally replicated samples were pooled except when genetically distinguishable (i.e. Rügen and Koldingfjord). Symbols: filled circle in orange, samples pooled into region 1 (North Sea), comprising autumn spawners (1–7), spring spawners (9–10) as well as winter spawners (8); plus, samples pooled into region 2 (Skagerrak), comprising spring spawners (11–13); filled triangle in green, samples pooled into region 3 (Kattegat and Western Baltic) comprising spring spawners (14, 15, 16_{02}, 16_{03}, 17, 18, 18_{MA02}). Hierarchical AMOVA estimates of variance: % variance among regions: 0.90 ($p<0.001$), % variance among populations within regions: 0.17. In the multi-dimensional scaling analysis we considered five dimensions. Dimensions 1 and 2 are plotted here; dimensions 3, 4 and 5 explained 4, 2.4 and 1.8% of the total variance, respectively.

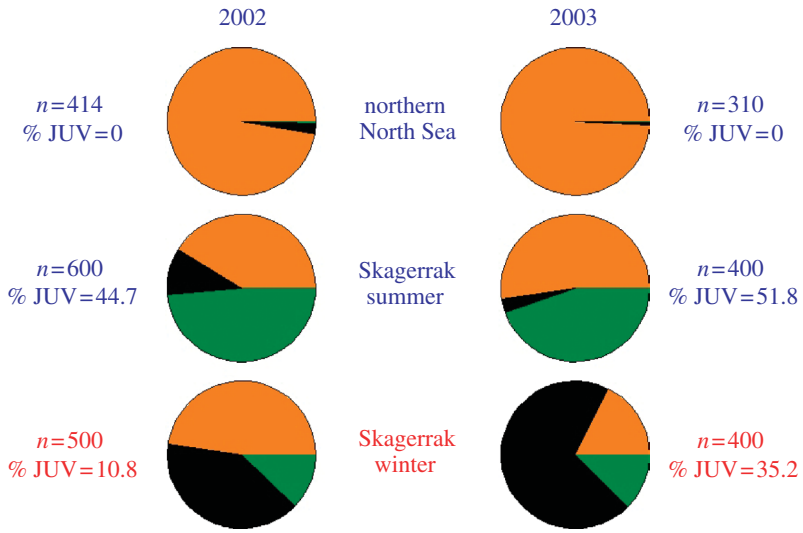

Figure 3. Estimated composition of mixture samples. Mixture samples in the NNS were collected in July of 2002 and 2003. In the Skagerrak mixture samples were collected in July and in November–December of 2002 and 2003 (details in electronic supplementary material, table A2). Composition of the mixture samples is reported in terms of herring from three broad regions: North Sea, (shaded in orange), comprising North Sea autumn spawners, English Channel winter spawners and Norwegian spring spawners; Skagerrak spring spawners: (shaded in black); and spring spawners from the Kattegat and Western Baltic (shaded in green), comprising spring spawners from the Kattegat, Inner Danish Waters and Rügen.

conducted by experienced technical personnel at the MARLAB (FRS) in the UK, at IMR in Norway and at DIFRES in Denmark. Otolith central area microstructure was analysed by IMR and DIFRES to determine each individual's hatching season (spring, autumn or winter; Moksness & Fossum 1991). Both age and hatching season (microstructure) were estimated following inter-laboratory calibration and repeated quality control provisions (details available in electronic supplementary material).

(d) *Statistical analysis*

Population differentiation was estimated per sample pair and overall using the unbiased F_{ST} estimator θ (Weir & Cockerham 1984) and statistical significance was examined using permutation tests implemented in FSTAT (Goudet 2001). Temporal, within-location samples not exhibiting significant differentiation (at $\alpha=0.05$) in these tests were pooled in subsequent analyses. Hierarchical AMOVAs were conducted using ARLEQUIN (Schneider *et al.* 2000).

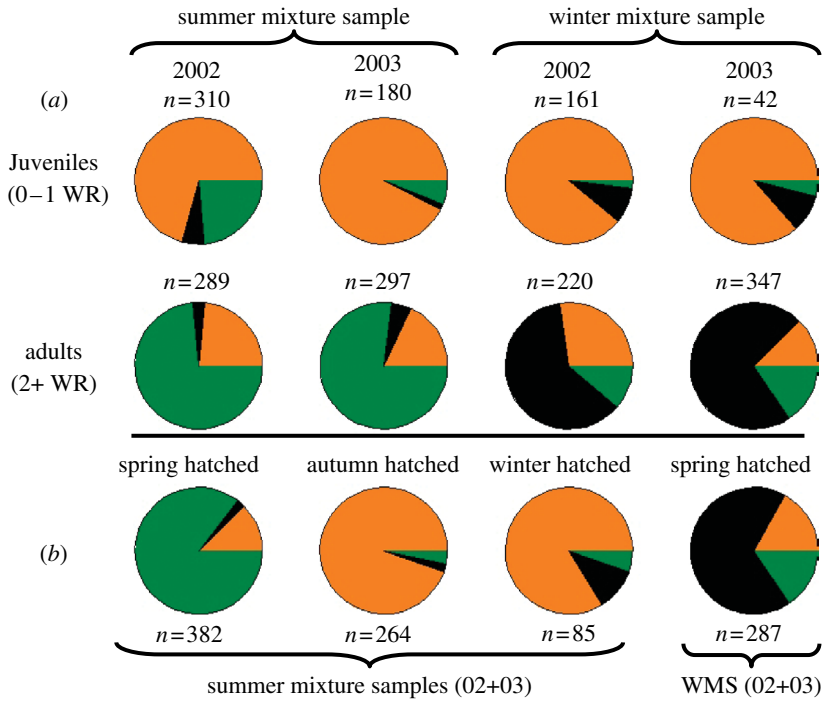

Figure 4. Estimated composition of subsets of the mixture samples where subsets are (*a*) age groups or (*b*) season hatched (as assessed from otolith microstructure). (*a*) Age group: individuals were pooled into two subsets. Juveniles, fish with no or one otolith winter ring; adults, fish with two or more otolith winter rings. This analysis was conducted only for the Skagerrak mixture samples as there were no juveniles present in the NNS mixture samples. Colours are as in figure 3. (*b*) Season hatched: individuals in the Skagerrak summer mixture samples from 2002 and 2003 were pooled into three subsets: spring hatched, autumn hatched and winter hatched. Only spring-hatched individuals were present in numbers sufficient for an MSA in the Skagerrak winter mixture samples from 2002 and 2003.

To examine the potential origin of herring collected from feeding aggregations in the NNS and in the SKG, we employed the partially Bayesian approach for the analysis of composition of stock mixtures developed by Pella & Masuda (2001). The software is available from the authors anonymous ftp site (ftp://ftp.afsc.noaa.gov/sida/mixture-analysis/bayes/). We followed the standard procedure as recommended in the MSA–BAYES user manual (Masuda 2002). In particular, for every computation, three independent Monte Carlo Markov chains were run, one per region (NS, SKG, WBS). Prior information on the proportions contributed by each of the three regions differed between runs. Computations were run for twice the number of iterations needed to achieve convergence, as assessed by two different diagnostics (see Masuda 2002). Estimates obtained before convergence were discarded; regional contributions are thus based on estimates obtained after convergence (Masuda 2002). MSA–BAYES was run independently for each of the six mixed aggregations' sample pools (NNS summer 2002 ($n=414$) and 2003 ($n=310$), SKG summer 2002 ($n=600$) and 2003 ($n=400$) and SKG winter 2002 ($n=500$) and 2003 ($n=400$; figure 3). MSA–BAYES was also run separately for herring in the mixture samples of different age-groups (juveniles (0 and 1 winter rings) and adults (2 and more winter rings)) and spawning season (spring, autumn, winter; figure 4).

(e) *Simulation tests of precision and accuracy*

Our baseline samples comprised temporally repeated collections of spawning herring collected from 18 locations distributed throughout the NS, the SKG and the WBS. We are, therefore, reasonably certain that these samples cover the spectrum of genetic variation of Atlantic herring in the area and that no important potential contributor to the mixed feeding aggregations was left un-sampled. The grouping of the baseline spawning herring collections into three relatively homogeneous regional pools (NS, SKG, WBS) provided the dual benefit of reducing the number of contributions to estimate, while simultaneously increasing the sample sizes within each baseline. Although the three regional stock-pools were genetically distinguishable (figure 2), the levels of differentiation were relatively low, hence, to gauge precision and accuracy of our estimates of mixture composition, we conducted a series of four simulations where mixture samples of $n=400$ were created with individuals randomly chosen in known proportions from the spawning aggregations sampled in 2002 and tested against three regional baselines based exclusively on allele frequency information for spawners collected in 2003. In the first simulation (electronic supplementary material, table A6) all three regional components contributed one-third each, whereas, contribution skewness increased in simulations 2–4, with each simulation differing in the details of which component contributed most. In general, precision and accuracy were high as indicated, respectively, by the relatively narrow 95% confidence intervals, and by the close relationship between the mean or median estimates and the true values. As expected, accuracy decreased with increasing skewness in the true composition of the simulated mixture samples, however, in all cases (except one where the true contribution was nil: simulation 3, region 2), the 95% confidence intervals encapsulated the true contributions.

3. RESULTS

We found that there are clear and temporally stable hitherto undetected genetic differences among herring

from three broad regions: (i) NS ($n=1332$), comprising NNS autumn spawners, English Channel winter spawners and Norwegian spring spawners, (ii) SKG ($n=635$), comprising SKG spring spawners and (iii) western Baltic (WBS, $n=1010$) comprising Kattegat and WBS spring spawners. These three regional components are characterized by relative homogeneity within and significant heterogeneity between them (two-level AMOVA, F_{CT}(differentiation among regions)$=0.009$, $p<0.001$; F_{SC}(differentiation among populations within regions)$=0.002$, $p<0.001$; figure 2).

These genetic differences, though generally low (maximum (mean) pairwise $F_{ST}=0.027$ (0.0085); see figure 2) as expected for an abundant, widely distributed and pelagic marine fish (Ward *et al.* 1994; DeWoody & Avise 2000; Palumbi 2003), are nevertheless of sufficient magnitude for a precise estimation of the regional contributions to mixed aggregations targeted by commercial fisheries in the NNS and SKG. To estimate such contributions, we employed a partially Bayesian approach for the analysis of stock mixtures (MSA–BAYES software; Pella & Masuda 2001; see electronic supplementary material).

Virtually all herring collected in July 2002 and 2003 within the NNS feeding aggregations likely originated in the NS with no contributions from SKG or the Kattegat and WBS (figure 3). The SKG feeding aggregations in contrast, unequivocally comprised herring of mixed origin: the summer feeding aggregations were composed partly of herring from the NS (mean estimated contribution 41.3% in 2002 and 52.4% in 2003) and partly from the Kattegat and WBS (mean 48.6% in 2002 and 44.7% in 2003; figure 3 and electronic supplementary material, table A4). Local SKG herring contributed only approximately 10% in 2002 and negligibly in 2003 (figure 3). The SKG winter mixture samples, which originated from locations closer to the coast of southeastern Norway (figure 1), exhibited a composition that was considerably different from that seen in summer samples: local SKG herring comprised 40 and 70% of the overwintering aggregations sampled in December 2002 and December 2003, respectively, with the remainder of the individuals originating from the NS (48% in 2002 and 18% in 2003) and the Kattegat and WBS (approximately 12–13% in both years; figure 3).

To examine the composition of the mixture samples in more detail, we next estimated contributions separately for juveniles (0–1 otolith winter rings) and adults (2 or more winter rings; figure 4, details in electronic supplementary material, table A5). This analysis was conducted exclusively for the SKG mixture samples as no juveniles were present in the NNS mixture collections. In both years (2002 and 2003) and both seasons (July and December) juveniles in the SKG mixed aggregations originated predominantly from the NS (figure 4; details in electronic supplementary material, table A5). SKG juveniles were virtually absent (figure 4), while the presence of juveniles from the WBS was relatively low throughout (figure 4; details in electronic supplementary material, table A5). This clearly indicates that the majority of juvenile herring in the SKG summer and winter aggregation samples are of NS origin.

The composition of adults within the SKG mixed aggregations differed from that of juveniles (figure 4). Within the summer (offshore) feeding aggregations most adults were of potential WBS origin with minimal contribution by NS herring in both years (figure 4). Within the overwintering (coastal) aggregations, most adults were of local SKG origin with remaining adults split between the NS and the WBS (figure 4; details in electronic supplementary material, table A5).

We inferred from otolith microstructure that each of the six pooled mixture samples (two in the NNS (2002 and 2003) and four in the SKG (2002 and 2003, summer and winter)) comprised subsets of individuals hatched in the spring, autumn and winter. Where sample sizes permitted ($n\geq85$; figure 4 and electronic supplementary material, table A5), we thus examined the potential origin of individuals within such subsets. Predictably, for the NNS mixture samples results did not change from those described above: virtually all herring hatched in the autumn and winter were of NS origin (see electronic supplementary material, table A5). Of the $n=382$ spring-hatched herring present in the SKG summer mixture samples, 84.5% (mean estimate) were likely of WBS origin, with only about 12% probably originating from somewhere in the NS. In contrast, of the $n=287$ spring-hatched herring present in the SKG winter mixture pool, 67.4% (mean estimate) likely originated locally in the SKG (figure 4). Among the autumn-hatched ($n=264$) and winter-hatched ($n=85$) herring in the SKG summer aggregations the vast majority of individuals was, predictably, of likely NS origin (figure 4).

4. DISCUSSION

Analysis of morphometric character differences on juveniles together with acoustic surveys and modelling studies (Iles & Sinclair 1982; Bartsch *et al.* 1989) suggest that larval and juvenile herring that hatch in the autumn and winter along the British NS coast drift across the northern and central NS into the SKG. These autumn-hatched juveniles spend the first year or two of life mixed with adult herring of local origin and with those of WBS origin on their annual feeding migrations into the Kattegat and SKG (Rosenberg & Palmén 1982; Aro 1989; ICES 1991; Johannessen & Moksness 1991). Historical records dating back to the tenth century (Alheit & Hagen 1997) suggest the degree to which NS herring intrude into the SKG correlates on decadal scales with certain climatic and hydrographic patterns. Our genetic results support a scenario of NS herring intruding into the SKG and mixing with local and WBS herring. Most importantly, they provide strong evidence for the persistence of genetic differences associated with life-history differences (spawning season, which is linked to spawning location, inferred from otolith central microstructure, migration pattern), among herring from three regions despite intermingling freely in large nursery, feeding and overwintering aggregations. The fact that such a complex pattern of intraspecific differentiation persists despite mixing supports the view of strong natal homing behaviour in herring (Iles & Sinclair 1982), at least at the broad geographic scale of our analysis.

Our results further suggest that the precise composition of the herring feeding aggregations in terms of the three major components varies both seasonally and spatially, a finding with profound implications for the conservation of genetic diversity in this species. We argue that sustainability (Hilborn *et al.* 2003), resistance to disturbance

(e.g. Hughes & Stachowicz 2004), and perhaps even the ability to recover from low abundance following environmental change or climatic extremes (e.g. Reusch *et al.* 2005), are all likely to be compromised if this genetic diversity is reduced through generalized management or misdirected area closures that can disproportionately impact smaller or less-productive populations. Loss of, or reduction in such biocomplexity is likely to have ecological implications by affecting the dispersal patterns that sustain major fisheries and evolutionary implications by removing adaptive genetic variation. We stress that detailed spatial and seasonal information is required for assessing the impact of spatially explicit conservation measures (e.g. marine protected areas, FSBI 2001), even for widely abundant and highly migratory species with low levels of genetic differentiation. Overlooking population differences in spatial use throughout their life cycles will affect the viability of populations, their ability to recover from low abundance and their evolutionary potential.

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Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area

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ABSTRACT: Determining spatio-temporal distributions of fish populations is of interest to marine ecology, in general, and to fisheries science in particular. Genetic mixed-stock analysis is routinely applied in several anadromous fishes for determining migratory routes and timing but has rarely been used for marine fishes, for which population differentiation is commonly weak and the method presumably less powerful. We used microsatellite information for Northeast Atlantic herring *Clupea harengus* L. populations and mixed stocks to address 2 questions. We used simulated mixture samples and 3 different statistical approaches to determine whether mixed stock composition could be determined with accuracy. Simulations showed that the applied approaches and mixture samples of 100 individuals enabled detailed composition analyses on a regional level, with resolution for tracing the ecologically dominant Rügen (Greifswalder Bodden) herring population. We then estimated spatio-temporal variation in herring migratory behaviour in the Skagerrak from 17 mixed samples collected over 2 seasons and 2 yr, and identified hitherto undescribed differences in distributions among populations that feed and winter in the area.

KEY WORDS: Genetic clustering · Genetic stock identification · GSI · Population structure · Simulation analysis · Skagerrak · Baltic Sea · Migration · *Clupea harengus*

INTRODUCTION

Understanding the dynamics of marine fishes requires knowledge about population level processes, and recent modeling studies demonstrate the importance of taking population specific exploitation rates into consideration in fisheries science and management (Kell et al. 2009, Kerr et al. 2010). However, inference about spatial distributions and exploitation of individual populations is greatly complicated when stocks are made up of fish from mixed population origin and are exploited disproportionately over time and space. Such heterogeneity is especially pertinent to population demography and management where respective populations differ in biological characteristics and/or abundance. Genetic mixed-

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stock analysis (MSA) comprises a number of statistical methods developed to estimate the composition of samples of individuals of mixed origin. MSA has been applied widely in anadromous salmonids (e.g. Ruzzante et al. 2004, Beacham et al. 2005, Koljonen et al. 2005, Smith et al. 2005, Gauthier-Ouellet et al. 2009, Miller et al. 2010), but in spite of the method's large potential for determining spatially and temporally explicit migratory behaviour, relatively few studies have yet been conducted in marine fishes (Waples & Naish 2009). One of the reasons for this paucity is that the generally modest levels of differentiation among marine populations (e.g. Ward et al. 1994) limit the statistical resolution for genetic stock identification (GSI), and, therefore, MSA (Manel et al. 2005, Waples & Gaggiotti 2006). However, when detailed sampling of the main populations contributing to mixed aggregations is attainable, approaches can be designed to overcome problems with low genetic resolution among populations (see e.g. Ruzzante et al. 2000, 2006).

Atlantic herring *Clupea harengus* L. is an abundant and widely distributed marine pelagic fish that spawns on substrate in coastal areas throughout most of the north Atlantic (Iles & Sinclair 1982). Most herring populations are migratory and often congregate on common feeding and wintering grounds where aggregations may consist of mixtures of individuals from several populations. One such area is found in the Skagerrak and eastern North Sea (ICES division IVaE). Here, mixed feeding aggregations generally comprise herring from the North Sea and the area spanning the transition zone between the North Sea and the Baltic Sea proper (here collectively referred to as the 'western Baltic Sea'). North Sea herring in the Skagerrak mainly constitute juveniles from populations spawning along the east coast of Britian and in the English Channel that drift as larvae into the Skagerrak, where they feed during their first 1 to 2 yr (Corten 1986, Johannessen & Moksness 1991). Western Baltic Sea herring in the Skagerrak comprise a presumably more diverse group of local populations. Main components represent: (1) adult herring that spawn in spring around the island of Rügen in the western Baltic and migrate annually to the Skagerrak to feed (Biester 1979, Aro 1989), (2) relatively small populations spawning in the Kattegat and inner Danish waters, and (3) local populations from the Skagerrak (Rosenberg & Palmén 1982). The Rügen herring are assumed to make up the majority of the western Baltic Sea herring in the area (ICES 2010). Although all population components are present in the Skagerrak during different times of the year, their spatial distributions and relative contributions to the mixed feeding and wintering aggregations remain unresolved (ICES 2010). Atlantic herring in the area thus comprise a good model system for examination of a relatively complex MSA scenario in an abundant marine fish.

Herring migratory patterns and habitat use in the Skagerrak have previously been studied based on population differences in morphological traits such as vertebral number, spawning time (spring, autumn or winter; estimated from otolith microstructure) and age distributions (review in ICES 2010). However, the statistical basis for assigning individuals to populations based on environmentally influenced traits that may exhibit large temporal variation remains uncertain (Bierman et al. 2010). Seminal microsatellite DNA studies demonstrate weak but significant genetic differentiation among populations within the Baltic Sea (Jørgensen et al. 2005a), among components in the transition zone between the North Sea and the Baltic Sea (Bekkevold et al. 2005), and among North Sea populations (Mariani et al. 2005). Ruzzante et al. (2006) used MSA with genetic baseline information obtained from those studies to estimate the proportions of herring originating from the North Sea, Skagerrak and western Baltic in samples from feeding grounds in the North Sea and Skagerrak. They pooled information for samples of feeding and wintering herring collected across the Skagerrak to show that otoliths-based estimates of hatching time generally correspond with the genetic origin of spring vs. autumn and winter spawning populations. The study also corroborated the prediction that juveniles feeding in the Skagerrak originate mainly from autumn and winter spawning populations in the North Sea and English Channel, whereas most herring older than 2 yr originate from spring spawning populations in the Skagerrak and western Baltic. However, resolving spatio-temporal aspects of contributions, e.g. with respect to which populations migrate within the Skagerrak have not previously been attempted. Here, we use genetic baseline information from the studies by Bekkevold et al. (2005) and Mariani et al. (2005) in a MSA. This analysis is targeted to determine spatial relationships of herring from the North Sea, Skagerrak, inner Danish waters, and Rügen in mixed fishery samples collected across SW-NE transects in the Skagerrak in both summer and winter, and repeated over 2 yr. Moreover, we use simulated mixed-stocks to assess the accuracy of the estimated stock compositions. Using a related MSA approach, Ruzzante et al. (2006) reported composition estimates for these mixture samples pooled

across all sampling locations using a different baseline designed to optimise that more general approach. Here, we also reanalyse their empirical data with the objective of estimating fine-scale spatial and temporal population differences in migratory behaviour and habitat use. We specifically seek to disentangle migratory patterns of the dominant Rügen population from those of populations from the Kattegat and inner Danish waters, which are assumed to be smaller and hence potentially more vulnerable to overexploitation.

MATERIALS AND METHODS

Samples of herring

Detailed information on the genetic composition of the baseline samples is reported in Bekkevold et al. (2005) and Mariani et al. (2005). Briefly, samples of spawning herring were obtained from 18

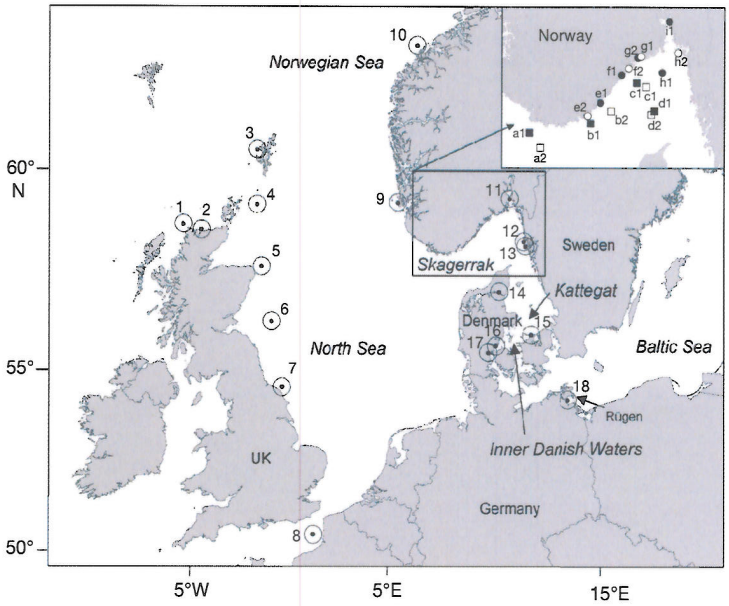

Fig. 1. *Clupea harengus*. Sampling locations for the genetic mixed-stock analysis baseline and mixed-stock collections (inset). Spawning location numbers refer to Table 1 and mixed-stock sample ID to Table 2. In inset, squares indicate summer samples, circles indicate winter samples and filled and open symbols indicate samples collected in 2002 and 2003, respectively

Table 1. *Clupea harengus*. Samples in genetic mixed-stock analysis baseline by reporting group. Locality numbers refer to Fig. 1. Details about samples are found in Mariani et al. (2005) for localities 1 to 8 and in Bekkevold et al. (2005) for localities 9 to 18. In simulations, the baseline was based solely on samples collected in 2003 and simulated mixed-stocks on samples collected in 2002, whereas the baseline used to analyse real mixed-stock samples comprised allele information for 2002 and 2003 samples combined within population (see 'Mixed-stock simulation analyses'). –: no data available

Reporting group	Sampling locality	Locality no.	Latitude/ Longitude	Sampling month	Sample size 2002	Sample size 2003	Spawning time
North Sea	Cape Wrath	1	58° 38′ N/5° 13′ W	August	84	96	Autumn
	Whiten Head	2	58° 36′ N/4° 20′ W	September	99	86	Autumn
	Shetland	3	60° 29′ N/1° 40′ W	August	90	–	Autumn
	Orkney	4	59° 12′ N/1° 40′ W	August	106	84	Autumn
	Aberdeen	5	57° 42′ N/1° 27′ W	August	–	91	Autumn
	Berwick	6	56° 18′ N/0° 58′ W	August	100	–	Autumn
	Flamborough	7	54° 34′ N/0° 27′ W	September	97	77	Autumn
	Downs	8	50° 7′ N/0° 25′ E	November	80	63	Winter
	Karmøy	9	59° 14′ N/05° 10′ E	March	100	–	Spring
	Møre	10	62° 78′ N/06° 08′ E	February	–	78	Spring
Skagerrak	Tjöme	11	59° 35′ N/10° 55′ E	March	120	116	Spring
	Måseskär	12	58° 32′ N/11° 32′ E	March	100	99	Spring
	Flatbrotten	13	58° 32′ N/11° 25′ E	March	100	100	Spring
Kattegat and Inner	Limfjord	14	57° 06′ N/10° 06′ E	May	–	99	Spring
Danish waters	Kattegat	15	55° 73′ N/11° 37′ E	May	44	99	Spring
(KIDW)	Kolding	16	55° 49′ N/09° 54′ E	April	100	70	Spring
	Lillebælt	17	55° 45′ N/09° 72′ E	April	–	100	Spring
Rügen	Rügen	18	54° 23′ N/13° 43′ E	March	100	–	Spring
				April	98	100	Spring
				May	100	100	Spring

locations widely distributed in the North Sea and adjacent areas. Baseline samples represent the majority of populations contributing migrants to the Skagerrak (Fig. 1). Of the 18 locations, 14 were re-sampled in 2 consecutive years to ascertain temporal stability of allele frequencies (Table 1). One location (Rügen) was sampled in March, April and May 2002 and in April and May 2003 to encompass potential effects of genetically differentiated spawning waves (Jørgensen et al. 2005b). Sagittal otolith growth patterns were used to estimate fish age (following procedures detailed in ICES 2003) and spawning season (spring, autumn or winter) following Clausen et al. (2007).

Data for mixed feeding aggregations were collected and analysed as described in Ruzzante et al. (2006). Briefly, herring were sampled by trawling along SW-NE transects in the Skagerrak during scientific surveys in summer and winter 2002 and 2003 (Fig. 1, Table 2). The age and hatching season were recorded for subsets of these fish following the same procedure as for spawning samples.

Molecular data

Baseline and mixed-stock individuals were genotyped for 9 tetra-nucleotide microsatellites as detailed in Bekkevold et al. (2005) and Mariani et al. (2005), and baseline allele frequency information was generated for each spawning location for each of the sampling years separately and for the combined information from replicate samples when preliminary analysis indicated no temporal allele frequency differentiation. Only temporal samples from locations 16 and 18 exhibited statistically significant variation and were treated separately throughout the analyses (Table 1). One microsatellite, locus *Cpa112*, exhibits genetic differentiation above neutral expectations (Larsson et al. 2007; Gaggiotti et al. 2009) and improves statistical power for population assignment (André et al. 2011). Genotype information was obtained for a total of 1900 individuals from 17 mixed fishery samples at high scoring success (average number of scored loci = 99% across loci and samples).

Mixed-stock simulation analyses

To compare the performance of different statistical approaches mixed-stock proportions were estimated using both a partly Bayesian approach implemented in the software BAYES (Pella & Masuda 2001) and the conditional maximum likelihood based ap-

Table 2. *Clupea harengus*. Mixed-stock samples (sample ID refers to Fig. 1). Numbers of fish which were determined from otoliths to have been spawned in spring (expected to originate mainly in the western Baltic), autumn and winter (expected to originate mainly in the North Sea and English Channel, respectively) are given per sample, along with proportion of juveniles (<2 yr, expected to mainly originate in the North Sea) in the sample. –: no data available

Location	Sample ID	Latitude/ Longitude	Sample date (dd/mm/yr)	Proportion juveniles	n	Spawning time			
						Spring	Autumn	Winter	Undetermined
Skagerrak West	a1	57° 53′ N/6° 35′ E	29/06/02	0.48	200	92	29	40	39
	a2	57° 41′ N/6° 55′ E	02/07/03	0.46	100	42	49	9	0
Central Skagerrak	b1	58° 2′ N/8° 22′ E	01/07/02	0.98	200	34	42	21	103
	b2	58° 13′ N/8° 57′ E	04/07/03	0.61	100	38	57	5	0
Skagerrak North	c1	58° 37′ N/9° 41′ E	03/07/02	0.13	100	26	3	0	71
	c2	58° 34′ N/9° 57′ E	06/07/03	0.23	100	73	22	3	2
Skagerrak South	d1	58° 10′ N/10° 7′ E	04/07/02	0.06	100	39	5	2	54
	d2	58° 10′ N/10° 5′ E	07/07/03	0.49	100	38	57	5	0
Grimstad	e1	58° 20′ N/8° 33′ E	17/12/02	0	100	100	0	0	0
Høvåg	e2	58° 8′ N/8° 16′ E	03/11/03	0	100	91	0	0	9
Risør	f1	58° 44′ N/9° 15′ E	02/11/02	0.62	100	–	–	–	100
Kragerø	f2	58° 49′ N/9° 27′ E	17/11/03	0.38	100	–	–	–	100
Langesund	g1	59° 0′ N/9° 48′ E	09/12/02	0.02	100	–	–	–	100
	g2	59° 0′ N/9° 48′ E	17/11/03	0	100	–	–	–	100
Inner Skagerrak	h1	58° 45′ N/10° 25′ E	04/11/02	1	100	–	–	–	100
	h2	59° 1′ N/10° 31′ E	19/11/03	0.04	100	96	1	0	3
Jeløya	i1	59° 29′ N/10° 37′ E	04/11/02	0.04	100	–	–	–	100

proach (Millar 1987) using the software ONCOR (S. Kalinowski, www.montana.edu/kalinowski/Software/ONCOR.htm). Both approaches offer the possibility to estimate contributions to the mixture sample both from individual populations in the baseline, as well as from user defined groups of populations, here referred to as 'reporting groups'. The latter is useful in analyses where regional population contributions are of interest and where modest or low genetic differentiation yields low statistical power for determining individual contributions to the mixtures. Here, initial simulations indicated low statistical power for estimating contributions by individual population components. Thus all analyses of mixed-stock composition were conducted using 4 geographically based reporting groups: (1) the North Sea, (2) the Skagerrak, (3) Kattegat and inner Danish waters (KIDW), and (4) Rügen. The 4 groups exhibit statistically significant structuring at varying degrees, with the largest pairwise F_{ST}s estimated between populations West and East of the transition zone, i.e. between the North Sea/Skagerrak and KIDW/Rügen populations (F_{ST}s between 1 and 2%), and with smaller degrees of differentiation within each side of the transition zone i.e. between North Sea and Skagerrak (~0.3%) and between KIDW and Rügen (~0.6%) (Ruzzante et al. 2006).

Two series of simulation analyses were conducted to examine the accuracy and bias of composition estimates generated. The first series was aimed at examining how varying the skew in proportions of contributing populations affected the estimates, and the second examined the effect of mixture sample size. In both cases, the simulation approach implemented in ONCOR was used to produce mixtures of 100 genotypes by drawing alleles from reduced variance estimates of allele frequencies in spawners collected in 2002 to make mixture samples with known population contributions. We first constructed 4 series of simulated mixture samples, where the skew among contributing populations varied with twenty replicates per skew scenario (Table 3). Twenty replicates per scenario enabled us to assess accuracy at a 5% level, which was considered adequate given the estimated accuracy that could be attained with the applied set-up (see 'Results'). Secondly, 4 series of mixed-stock samples of 50, 100, 150 and 200 multi-locus genotypes were constructed, with North Sea, Skagerrak, KIDW and Rügen contributing 40, 5, 25 and 30%, respectively, so as to simulate a relatively complex mixture scenario. Again, twenty replicates were produced for each sample size. The composition of simulated mixtures was estimated using baselines constructed with allele frequency information from herring collected in 2003. Constructing the baseline samples using the allele frequency distribution of the 2003 collection to estimate the composition of simulated mixture samples based on the allele frequencies of 2002 collections, ensures that allele frequency estimates in simulated mixture samples are independent of allele frequency estimates in baseline samples. Absence of this independence would be expected to lead to upward biasing of predictions about accuracy (e.g. Anderson et al. 2008). BAYES was run following standard procedures recommended in the user manual (Masuda 2002). Briefly, each mixture was analysed using 4 independent MCMC chains, run until convergence, as assessed by the Raftery & Lewis (1996) diagnostic. Individual chains were started with 95% of the mixed sample initially contributed by one source population, and the rest were divided equally among the remaining populations. Across the 4 chains, all 4 reporting groups were represented as initially dominating. The Dirichlet prior distribution was set as a low information prior giving all population proportions equal weights. Convergence among chains was assessed with the Gelman & Rubin (1992) diagnostic. Point estimates (means) and 95% probability intervals (95%PI) for posterior MCMC samples of reporting groups were determined using only the second halves of chains combined (MCMC sample size 10 000 per chain). ONCOR was run, using standard settings and 95% confidence intervals (abbreviated 95%CI) were assessed by 10 000 bootstraps. Accuracy and bias of the estimates generated across simulations with ONCOR and BAYES were evaluated by the average absolute deviation between true and estimated proportions and by the mean square error (RMSE) and the relative bias (RBias).

For comparison, we also estimated MSA accuracy for the 4 reporting groups using the software BELS (Bromaghin 2008). In BELS, simulated mixtures of user specified size and population compositions are produced from baseline data by sampling with replacement single-locus genotypes from baseline individuals. This step is followed by MSA using conditional maximum likelihood to generate estimates of individual populations that are summed across populations within reporting groups. Using BELS we simulated mixture compositions with the same degrees of skew and sample sizes as simulations in Table 2, and replicated each scenario 1000 times. The approach thus enables analysis of a very large number of simulated samples.

Table 3. *Clupea harengus*. Mixed-stock estimates for simulated mixture samples for the 4 reporting groups defined in Table 1. True proportions and their estimates in BAYES and ONCOR (average estimates across 20 replicates per scenario), with 95% probability intervals or 95% confidence intervals (averaged across 20 replicates) are given together with the RMSE and RBias for each scenario. Also shown are results for analyses performed in BELS (Bromaghin 2008). In the latter, estimates represent the average proportion estimated across 1000 re-sampled mixtures. See 'Mixed-stock simulation analysis' for details

Simulated scenario	Reporting group	True proportion	BAYES				ONCOR				BELS
			Estimated proportion (average)	95%PI (average)	RMSE	RBias	Estimated proportion	95%CI (average) (average)	RMSE	RBias	Estimated proportion (average)
Sim1					0.34	−0.03			0.11	0.00	
(no skew)	North Sea	0.25	0.28	0.12–0.47			0.26	0.14–0.44			0.31
	Skagerrak	0.25	0.21	0.03–0.42			0.29	0.10–0.46			0.18
	KIDW	0.25	0.13	0–0.45			0.23	0.10–0.43			0.3
	Rügen	0.25	0.34	0.13–0.57			0.23	0.05–0.35			0.22
Sim2					0.38	−0.04			0.31	0.12	
(low skew)	North Sea	0.50	0.49	0.28–0.69			0.38	0.22–0.56			0.49
	Skagerrak	0.17	0.14	0.01–0.41			0.30	0.11–0.47			0.15
	KIDW	0.17	0.06	0–0.26			0.15	0.05–034			0.23
	Rügen	0.17	0.28	0.10–0.45			0.17	0.02–0.29			0.13
Sim3					0.08	−0.17			0.15	−0.38	
(Rügen skew)	North Sea	0.25	0.17	0.06–0.30			0.16	0.07–0.30			0.28
	Skagerrak	0	0.06	0.01–0.21			0.15	0.03–0.30			0.02
	KIDW	0	0.00	0–0.10			0.23	0.12–0.48			0.09
	Rügen	0.75	0.74	0.60–0.86			0.47	0.20–0.56			0.61
Sim4					0.33	−0.22			1.59	0.51	
(North Sea skew)	North Sea	0.60	0.60	0.40–0.75			0.46	0.27–0.61			0.57
	Skagerrak	0.10	0.06	0–0.28			0.22	0.07–0.42			0.08
	KIDW	0.05	0.02	0–0.18			0.11	0.03–0.29			0.15
	Rügen	0.25	0.29	0.12–0.45			0.21	0.04–0.32			
Across simulations					0.28	−0.09			0.52	0.11	0.2

Skagerrak mixed-stock fishery samples

MSAs were carried out for each of 17 mixture samples, estimating contributions from the North Sea, Skagerrak, KIDW and Rügen using both BAYES and ONCOR. The baseline in these analyses comprised allele frequency information for all population samples, with temporal information combined within locations when applicable (i.e. including the full dataset in Table 1, with temporal samples from location 16, and March and combined April and May samples from location 18 entered as separate population samples). Analyses were performed using the same procedures as for simulated samples.

RESULTS

Composition estimates for simulated samples from BAYES and ONCOR generally returned estimates close to true proportions, although across individual simulations, compositions were over- or underestimated by up to 24% (BAYES) and 41% (ONCOR) (Table 3). BAYES estimates were overall more accurate and less biased than ONCOR estimates (RMSE = 0.28 and 0.52 for BAYES and ONCOR, respectively; RBias = −0.09 and 0.11 for BAYES and ONCOR, respectively). ONCOR tended to overestimate weakly contributing reporting groups and underestimate strongly contributing reporting groups, whereas BAYES displayed relatively low accuracy under the no-skew scenario (Table 3). Across simulations, average deviation between true and estimated proportion was 8% for BAYES and 11% for ONCOR. The simulations suggested that ONCOR and BAYES, respectively, tended to under- and overestimate propor-

tions for Rügen, but that BAYES produced overall less biased results (Table 3). Confidence and probability intervals were generally broad for both BAYES and ONCOR (average ~0.30 for both approaches) and sometimes included zero for groups contributing up to 17% of the mixtures. 95%CIs for ONCOR sometimes did not include the true values or even the composition estimates (ONCOR authors note that this can be a problem). The 95%PIs and 95%CIs for non-contributing groups were narrow and always included zero in both BAYES and ONCOR. BELS analyses also indicated that contributions from the 4 reporting groups could be estimated with acceptable accuracy, as deviations between real and estimated proportions were generally low (average = 0.05) (Table 3). For simulated mixtures of different sample sizes BAYES results indicated that 95%PIs decreased with increasing sample sizes, whereas average point estimates changed little and on average deviated from actual values by 0.12, 0.07, 0.05 and 0.06 for sample sizes of 50, 100, 150 and 200, respectively (Table 4). Similar BELS simulations confirmed this result (data not shown).

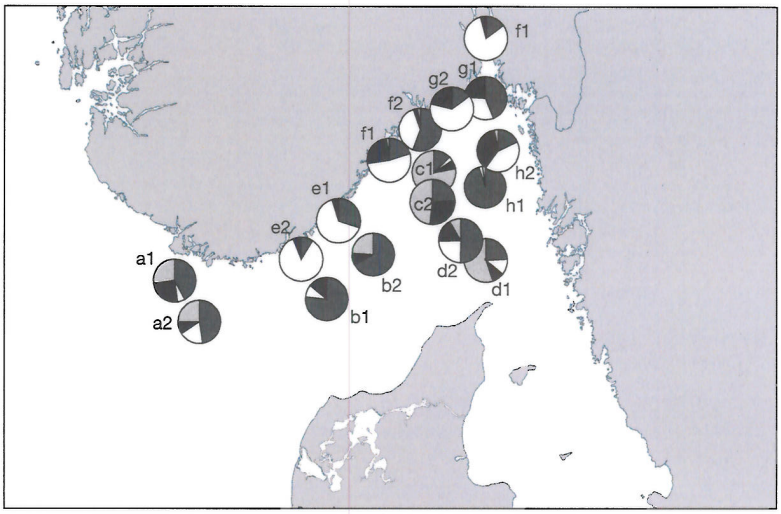

Fig. 2. *Clupea harengus*. BAYES estimates of proportional contributions from each of the reporting groups North Sea (dark grey in pie diagram), Skagerrak (white pie), KIDW (black pie) and Rügen (light grey pie) to 17 mixed-stock samples collected across 2 yr (sample numbers refer to Fig. 1 and Table 2, sample notations '1' and '2' indicate samples collected in 2002 and 2003, respectively; samples a–d were collected in summer and e–i in winter)

Skagerrak mixed-stocks

The simulation analyses indicated that BAYES and ONCOR produced mixed-stock proportions at roughly similar levels of accuracy, with BAYES performing slightly better under skew. All 17 empirical samples exhibited evidence of skewed contributions (see below), and, therefore, we only report BAYES estimates. When MSA results for the 17 fishery samples from locations a to i (Fig. 2) were compared, 3 main patterns emerged. First, herring of local Skagerrak origin were indicated to be present in very low numbers in most summer samples (a to d), and in 7 out of 8 samples lower 95%PI included zero. Second, in stark contrast to this, local Skagerrak herring made up substantial proportions in samples collected in winter (e to i). Results indicated that Skagerrak herring often occurred together with North Sea herring (juveniles) or completely dominated the aggregations they were collected from, and only rarely schooled with fish originating in KIDW and Rügen. Third, whereas Rügen herring were found in all sum-

Table 4. *Clupea harengus*. Mixed-stock estimates for simulated mixtures, computed with BAYES for varying sample sizes (n) (reported as averages across 20 replicates per n) with average 95% probability interval (95%PI) and RMSE and RBias

Reporting group	True proportion	n = 50		n = 100		n = 150		n = 200	
		Proportion	95%PI	Proportion	95%PI	Proportion	95%PI	Proportion	95%PI
North Sea	0.40	0.32	0.12–0.52	0.36	0.16–0.47	0.36	0.25–0.50	0.39	0.27–0.50
Skagerrak	0.05	0.23	0.04–0.50	0.10	0.08–0.43	0.11	0.05–0.30	0.10	0.08–0.33
KIDW	0.25	0.18	0.04–0.45	0.25	0.07–0.38	0.23	0.06–0.28	0.17	0.07–0.25
Rügen	0.30	0.27	0.03–0.42	0.29	0.08–0.39	0.30	0.18–0.41	0.38	0.17–0.36
RMSE		4.48		1.69		1.34		1.11	
RBias		–0.03		0.08		–0.14		0.08	

mer samples and often made up the dominant part of western Baltic herring, they were estimated at very low proportions in all winter samples (all estimates below 3%) and lower 95%PIs always included zero. North Sea herring were indicated in all collections, and were mostly with spring-spawning herring. Only one sample (h1) showed evidence of being completely dominated by North Sea herring (Fig. 2). Across the 2 yr, contributions from North Sea and Skagerrak population varied relatively little between samples from locations at a, b and c, whereas contributions from Rügen and KIDW varied considerably across all locations (Fig. 2). Apart from the fact that local Skagerrak herring dominated most samples, composition estimates for winter samples generally varied between years within locations, indicating no obvious trend for spatially differentiated habitat use among population components from the North Sea, KIDW, and Rügen within a given season.

Overall the MSA results corresponded with expectations from otolith estimated hatching season and age compositions, in that there were positive relationships between estimated contribution from North Sea populations in a sample and both proportions of juveniles (Spearman's rank correlation $r_S = 0.81$) and proportions of herring estimated to have hatched in autumn or winter ($r_S = 0.90$) (compare Table 2 and Fig. 2). Nonetheless, comparisons also showed that some samples (f1) dominated by juveniles had low contributions from North Sea herring, whereas other samples (g1, d1, e1) with estimated contributions from the North Sea between 24 and 44% contained no or few (<6%) juveniles. In winter samples, a positive relationship was found between proportions of adult and local Skagerrak herring ($r_S = 0.70$). Collectively, winter samples comprised only 19 juveniles out of 281 spring-spawned individuals (as assessed from otoliths), and a *post hoc* MSA of the origin of 74 spring-spawned juveniles encountered across summer samples estimated that only 1% (95%PI 0–23%) originated from the Skagerrak, whereas 21% (95%PI 6–38%) were from the North Sea, 14% (95%PI 0–36%) from KIDW, and 60% (95%PI 37–80%) from Rügen.

DISCUSSION

The simulation analyses returned 2 main results. First, the applied statistical approaches in which population samples are grouped present useful tools for investigating habitat use and migratory behaviour for weakly differentiated populations, even across small spatial scales. Secondly, our simulations demonstrated that MSA can estimate contributions from specific populations, even in a weakly differentiated population scenario (in our case Rügen herring). Nonetheless, composition estimates deviated, on average, by almost 10% from true proportions indicating that the MSA results should be treated qualitatively, rather than quantitatively. Moreover, conclusive inference about presence/ absence of rare populations was not possible with the current design, as evidenced by the fact that, although non-contributing reporting groups came out with very low estimates, contributions of up to 17% to a mixture were associated with lower CIs including zero (Table 3). We found that the Bayesian method performed better than the conditional maximum likelihood method, which is in agreement with the expectation when highly polymorphic loci are used (e.g. Kalinowski 2004). For BAYES, the simulations indicated that for groups contributing more than 25% to a mixture, contribution estimates never deviated more than 10% from the true proportions. For ONCOR, estimates for groups contributing more than 25% regularly deviated by 20 to 40%, and only in the (presumably biologically rare) no-skew scenario did ONCOR perform better than BAYES (Table 3). Simulation analyses hence indicated that BAYES estimates could be used to reliably detect major contributions from the 4 reporting groups and to indicate if reporting group contributions were rare or absent.

Simulation analyses are unlikely to completely predict MSA accuracy as sampling variance, temporal allele frequency variation, and violation of model assumptions may lead to error. A well known problem arises from using simulated mixture files constructed by re-sampling baseline genotypes, leading to underestimation of sampling variance, and to a corresponding overestimation of MSA accuracy (Anderson et al. 2008). Here, temporally replicated samples from baseline populations were available, allowing construction of baselines and simulated mixture files with independent genotypic information, thus minimising effects of sampling error on MSA predictions. The approach, however, does not incorporate the full information available, as allele frequency estimates are expected to improve with increased sample sizes. All our population samples were large overall (ca. 100 ind.), indicating that effects of sampling error on allele frequency estimates are unlikely to have been a major source of bias when estimating contributions of reporting groups to mixtures. Nonetheless, sampling error may have contributed to the fact that BAYES and ONCOR estimates were gener-

ally more similar across empirical mixed-stocks (r_S = 0.97) than across simulated mixtures (r_S = 0.75), where baseline allele frequencies for the latter were estimated based on approx. half the number of individuals, compared to the former. It is thus expected that the simulations yield conservative estimates of MSA accuracy. In contrast to simulated mixtures, real mixed samples may contain individuals originating from unsampled, genetically differentiated populations. Estimation errors associated with occurrence of mixture individuals from unsampled baselines were initially addressed by Smouse et al. (1990) and Pella & Masuda (2006) who suggested that the problem may be reduced when unsampled populations have some genetic similarity with populations in the baseline, as is expected under isolation-by-distance. Although our baselines were expected to comprise samples from all major population components in the area, the distribution of herring spawning sites is more or less continuous in the study area, and we are unlikely to have sampled all genetically distinct components contributing to mixed samples. However, the fact that 95%PIs differed little between simulated and empirical samples (average PI: Sim1 to 4 = 0.309; samples a1 to i1 = 0.286) suggested that the overall resolution of simulated and empirical MSAs was similar and that contribution from unsampled populations was not likely to be a major error source. Due to logistic and resource constraints, fully comprehensive population sampling is generally unlikely to be attainable in most marine MSA applications. Our results thus demonstrate that even though failure to sample all baseline populations may affect MSA accuracy, sampling error need not pose a problem for regional estimates.

Population allele frequencies may vary over time, e.g. through random genetic drift among cohorts (Jorde & Ryman 1995) or as an artifact of inconsistent sampling of sub-structured populations, and this may pose another source of error in MSA applications. Such effects are, however, expected to contribute little to allele frequency variation in marine organisms with extensive overlap between generations and the presumed very large effective population sizes. Our analysis comprised populations exhibiting lower differentiation than MSA studies in most other marine species (Wirgin et al. 1997, Ruzzante et al. 2000, 2006, Beacham et al. 2008, Wennevik et al. 2008) and approximately an order of magnitude lower than MSA studies in salmonids (Ruzzante et al. 2004, Beacham et al. 2005, Koljonen et al. 2005, Smith et al. 2005, Gauthier-Ouellet et al. 2009). Using BAYES with data for 8 microsatellite loci typed in 26 Atlantic salmon populations, Koljonen et al. (2005) reported that deviation between true and estimated single population contributions averaged 3% in a composite self-assignment test (i.e. simulated mix-samples consisted of multi-locus genotypes re-sampled from the baseline). Although their study estimated population- (and not region-) specific mixed-stock contributions, the comparable levels of accuracy observed in their study and ours emphasize that even at low population differentiation, as e.g. F_{ST} < 0.02, MSA accuracy may be within acceptable limits for biological inference.

Kalinowski (2004) used a fully simulated dataset to examine performance of maximum-likelihood based MSA under various levels of marker polymorphism and baseline sample sizes. He found that maximal MSA accuracy was obtained by analysing ca. 100 ind. per baseline population using markers with a high number of independent alleles per locus (in the range of ca. 20 to 250) and that accuracy was independent of levels of population differentiation for F_{ST}s ranging between 0.01 and 0.16. These conditions were predicted to apply to the BAYES approach also (Kalinowski 2004). Very high polymorphism and allelic richness are typical for microsatellite marker studies in marine fishes and also in our study (heterozygosity and numbers of alleles per locus averaged across samples and loci were 0.820 and 26, respectively). Our approach and sampling scheme should thus yield close to maximal statistical power with the given markers, and the results can be used to predict power in other marine MSA applications exhibiting similar levels of differentiation and with similar combinations of markers and sample sizes.

The accuracy of MSA estimates is potentially affected by the composition in the mixed-stock, as well as coverage of the baseline. Mixtures exhibiting low contribution skew (i.e. all components present contribute similar proportions) are expected to return better estimates than mixtures with high skew in relative population contributions (Koljonen et al. 2005). Contributions from lesser components tend to be overestimated with most algorithms, with corresponding underestimation of larger proportions (e.g. Reynolds & Templin 2004). With the Bayesian method we did not observe that simulations with lower skew among contributing components exhibited higher accuracy, and rare components were both over- and underestimated (Table 3). In contrast, ONCOR produced overall more biased estimates, and in several cases overestimated contributions from low- or non-contributing reporting groups by more than 10%.

Varying sample sizes of simulated mixtures mainly affected 95%PI and only to a small extent composition point estimates (Table 4), showing that sample sizes of 100 were adequate for inference about composition of the Skagerrak fishery samples. Point estimates for the Skagerrak reporting group contributing 5% approached zero in all cases, showing that power for detecting presence/absence of rare components did not increase significantly even with a fourfold increase in sample size.

Re-analysis of stock composition for the 17 feeding samples that were pooled and analysed for a different set of reporting groups in Ruzzante et al. (2006) generated novel information about herring migratory behaviour. The tendency for samples to show some stability in composition across the 2 sampling years suggests stable spatial structure and habitat use of individual population components (Fig. 2). This was illustrated by western locations a and b comprising high proportions of North Sea fish in both years, whereas a relatively strong dominance of Rügen fish was observed for eastern locations c and d in 2002. At d in 2003, juvenile North Sea herring dominated together with local Skagerrak herring. Variance in composition among schools sampled in the same general area and the same time of the year is expected to be large, and our sampling scheme is unlikely to be adequate for fully resolving population differences in habitat use in the study area. Our analysis nonetheless demonstrated that local Skagerrak fish were present and often dominated aggregations sampled in winter. Winter samples were generally collected closer to the coast than summer samples, and we are thus not able to discount that the large seasonal differences in compositions could be an effect of coastal vs. offshore distributions of populations. The one deviation from dominance of local Skagerrak herring in winter samples was in fact the h1 sample of juveniles exclusively of North Sea origin, which incidentally was also the winter sample collected furthest offshore. However, local origin Skagerrak herring were also indicated to dominate the offshore winter sample h2, and our data clearly show that Skagerrak fish were present also in samples collected offshore in summer, and thus that spatial segregation of local herring is not just a question of inshore or offshore habitat.

There was generally good correspondence between estimated ages, otolith-assessed spawning times, and sample compositions, supporting the assumption that North Sea herring in the area are mainly represented by juveniles hatched in autumn or winter, whereas western Baltic herring in the area are mainly spring-spawning adults (Rosenberg & Palmén 1982). Previous attempts to infer migratory behaviour from catch statistics separated according to age groups and morphmetrics have failed to reliably distinguish among populations from the western Baltic (e.g. from the Rügen vs. other western Baltic populations) and in some cases even among herring from the Norwegian Sea and the Baltic Seas) (e.g. Payne et al. 2009). The efficiency of morphometric methods to reliably distinguish among populations is likely to vary among years in response to changes in environment, some of which are known to affect morphological traits (e.g. Bierman et al. 2010). Similarly, spawning time, as assessed from otoliths, is not a reliable indicator of population origin, as spawning time can vary within populations (e.g. Bekkevold et al. 2007).

Here, using a suite of 9 microsatellite loci we were able to gain novel insights at lower structural levels. We found no evidence that Skagerrak juveniles feed or overwinter in any of the sampled areas, which, incidentally, are important feeding and overwintering habitat for juvenile North Sea herring. Neither the location of juvenile Skagerrak herring feeding grounds nor the spatial segregation between juvenile and adult Skagerrak herring have, to our knowledge, been described. However, spatial segregation among juveniles from individual spring-spawning populations may facilitate subsequent natal homing (Corten 2001, Gaggiotti et al. 2009) and thus contribute to the relatively large reproductive isolation between populations from the Skagerrak and KIDW (Bekkevold et al. 2005). The relatively high proportion of spring-spawned juveniles of genetic North Sea origin is interesting. These individuals could potentially represent juveniles from the Norwegian Sea spring-spawning population that cannot be genetically distinguished from North Sea autumn-spawning herring with the applied set of markers (Mariani et al. 2005). The observation that some samples with no or few juveniles and no or few autumn- or winter-spawning individuals (d1, e1 and g1) contained substantial proportions of North Sea herring could also suggest migration of Norwegian Sea spring-spawning herring. However, feeding migration of Norwegian herring to the Skagerrak has not been described and resolving their origin requires more targeted analyses involving markers that allow separation of North Sea and Norwegian populations.

We found that contrary to previous understanding (e.g. Rosenberg & Palmén 1982) herring spawning in the KIDW were found to be present in considerable numbers throughout the Skagerrak. Pooling across

spatial samples and years, their contributions were estimated at 14% in summer and 13% in winter, translating into respective contributions of 24 and 20% of all (spring-spawning) western Baltic herring in the Skagerrak. Current fisheries management advise for TAC for the Skagerrak mixed fishery is set at a level compatible with a precautionary exploitation of western Baltic Sea herring and is based on the assumption that Rügen herring constitute the majority of spring-spawning herring in the area (ICES 2010). Although our results support the notion that Rügen herring constitute an important component in the Skagerrak, they also show that the proportion of spring-spawning herring in the Skagerrak represents an inaccurate proxy for the size of the Rügen component.

In summer, Rügen fish contributed substantially to most collections. However, in winter they were absent from all collections, in agreement with inference from mark-recapture studies that Rügen herring leave their western Baltic spawning grounds in the 2nd quarter of the year to feed in the Skagerrak and eastern North Sea in summer, followed by return to spawning locations in the following 1st quarter (Biester 1979, Nielsen et al. 2001, van Deurs & Ramkaer 2007, Payne et al. 2009). The fact that the Rügen herring were rare or absent from all our November samples collected across 2 years suggests that their return migration may have started before, or during the 4th quarter. This pattern also supports the assertion from morphometric and otolith studies that Rügen herring overwinter south of the Skagerrak, in the western Baltic (Rosenberg & Palmén 1982, Nielsen et al. 2001). In contrast, several winter samples contained Kattegat herring and herring from the inner Danish waters in considerable proportions (compare Fig. 2 samples a to d with e to i). We interpret these results as indicating that habitat use and migratory behaviour differ profoundly between the Rügen and KIDW herring. As mentioned, summer samples were collected offshore and most winter samples were collected inshore (Fig. 1), and we therefore cannot directly describe the seasonal variation in mixed-stock composition. However, we identified large differences in habitat use and migratory behaviour among (spring) spawning components, demonstrating that even for organisms exhibiting weak population differentiation, the MSA approach is a powerful tool that can be tailored to answering specific biological and management questions. In the case of the Rügen herring, the current assessment procedure assumes that Rügen is the strongest contributor to the spring spawning stock occurring throughout the area spanning from the eastern North Sea to the western Baltic, and uses a recruitment estimate based solely on this population (ICES 2010). However, our findings suggest that including recruitment estimates on a more local population scale would reduce noise around this estimate. Our results thus also strongly support the notion (e.g. Schindler et al. 2010) that marine fish management needs to incorporate knowledge about individual population dynamics to allow sustainable exploitation.

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Effect of spatial differences in growth on distribution of seasonally co-occurring herring *Clupea harengus* stocks

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RUNNING HEADLINE

GROWTH AND DISTRIBUTION OF HERRING

ABSTRACT

The mechanisms most likely to determine the distribution of the two major herring *Clupea harengus* stocks in their common early summer feeding ground in the Eastern North Sea, Skagerrak and Kattegat were investigated through analysis of acoustic survey data from six consecutive years. No change was detected in biomass of North Sea Autumn Spawning *C. harengus* (NSAS) over time whereas the biomass of Western Baltic Spring Spawning *C. harengus* (WBSS) severely declined. Analyses of centre of gravity by stock showed no change in NSAS distribution, whereas the WBSS changed to a more western distribution over time. Contrary to previous perception of the juvenile migration, NSAS were found to leave the study area already at the age between 1 and 2 and WBSS 1 year olds were encountered in the Skagerrak. The estimated parameters of von Bertalanffy growth equations showed marked differences between areas with fish in the eastern part of the area having the lowest size at age at all ages. Further, their growth conditions appeared to deteriorate progressively over the period studied. Both NSAS and WBSS showed the highest condition in the North Sea and Skagerrak while condition was substantially lower in Kattegat. The westward movement of spring spawners over time suggests that growth rate and possibly density of conspecifics influences the migration pattern and distribution of *C. harengus* in the area. In contrast, there was no evidence to suggest that distribution was constant over time within stocks or that distribution reflected size dependent limitations on migration distance.

KEY WORDS: Acoustic surveys, Skagerrak, condition, migration, stock identity

INTRODUCTION

Atlantic herring *Clupea harengus* (L.) exhibit marked diversity over their distribution range, often showing complex population structures with both genotypic and phenotypic variation (Bekkevold *et al*., 2005; Ruzzante *et al*., 2006) and a wide variety of migration patterns and growth forms (Secor *et al*., 2009; Brunel & Dickey-Collas, 2010). Often two or more *C. harengus* stocks are targeted by a single fishery exploiting shoals of mixed population origin (Rosenberg & Palmén, 1982; Clausen *et al*., 2007a; Payne *et al*., 2009) and population-specific exploitation rates may vary strongly both within and between years in response to combined effects of the spatial distribution of the fishery, spatio-temporal variation in the degree of population mixing and the relative biomass of the individual populations (Bekkevold *et al*., 2011). The degree of mixing between stocks is often variable and unpredictable which challenges successful spatio-temporal fishery management, particularly when populations are asynchronous in population dynamics (Payne *et al*., 2009). In these cases, it may be necessary to restrict fishing on one stock while the other stock can potentially sustain a larger fishing pressure. Managing fisheries of distinct *C. harengus* stocks is essential for several reasons: to maintain yields in the area, and to avoid stock depletion of the weaker component; and also to ensure the unique ecosystem function of *C. harengus* remains intact. One possibility is to use spatial management measures (Kell *et al*., 2009), but in order for this to be successful, knowledge of what determines the migratory behaviour and also the degree of mixing in different areas is essential.

C. harengus in the Skagerrak, the Kattegat and the Western Baltic (Figure 1) consist of a mixture of migrating populations with different life history characteristics. Of these, the populations within the North Sea Autumn Spawner stock (NSAS) and Western Baltic Spring Spawner stock (WBSS) are dominating summer foraging aggregations (Bekkevold *et al*., 2011). The two *C. harengus* stocks are

52 targeted by a fishery in the Skagerrak and the Kattegat as well as the eastern parts of the North Sea
53 exploiting shoals of mixed population origin (Rosenberg & Palmén, 1982; Payne *et al*., 2009; Clausen
54 *et al*., 2007b). The two populations follow specific migration patterns. Spawning of the WBSS occurs
55 in February-May with the most important spawning ground at the Greifswalder Bodden off the island
56 of Rügen (Biester 1979) where hydrographical retention keep larvae near local nursery areas in the
57 Western Baltic sea (Polte *et al.,* 2013). The majority of the 2+ winter ring WBSS *C. harengus* are
58 assumed to migrate out of the subdivision 24 for the summer feeding grounds in Division IIIa and the
59 eastern North Sea (Payne *et al.,* 2009). During autumn, the WBSS return to the southern part of the
60 Kattegat with the majority of the stock overwintering in the Sound (ICES subdivision 23) (Biester,
61 1979; Otterlind, 1987; Nielsen *et al*., 2001). NSAS *C. harengus* larvae, hatched in autumn along the
62 UK east coast and in winter in the English Channel, drift from the spawning areas to subsequently
63 metamorphose in spring near the nursery area ranging from the eastern North Sea into to the Skagerrak
64 and the Kattegat (Burd, 1978; Heath *et al*., 1997). NSAS are assumed to remain in this area until 2
65 winter ring when they start to mature and join the adult population feeding aggregation in the central
66 and northern North Sea (Corten, 1986).

67 Atlantic *C. harengus* populations are often highly migratory with migration distance varying from a
68 few 100 km to more than 1000 km (Slotte, 1999; Alerstam *et al*., 2003). These migrations are assumed
69 to be adaptations to the local environmental conditions for increased success in spawning, growth or
70 survival of offspring and/or maturing individuals. Migration take advantage of spatial and temporal
71 differences in the distribution of resources (being food, spawning habitat availability, shelter for
72 predators, etc.), and thus increase the fitness of the migrants (Harden Jones 1968; Chapman *et al*.,
73 2012). For such behaviour to evolve, the benefits of using two or more different areas during a defined

74 time-cycle must outweigh the costs of the migration. *C. harengus* may use predictive (genetic factors or
75 learning) and reactive (response to near field or state-space comparisons) orientation mechanisms
76 during migration (Harden Jones 1968, Fernö *et al*., 1998). A combination of reactive and predictive
77 orientation mechanisms may provide *C. harengus* with a flexible migration strategy, adapted to both
78 predictable and unpredictable conditions (*Fernö et al*., 1998). The underlying behavioural mechanism
79 could be influenced by ‘‘enviroregulation’’, as suggested for *scombrids* (Reid *et al*., 1997), where the
80 fish select their immediate environments by swimming towards ‘preferred’ environmental conditions.
81 For *C.harengus* it has been shown that the intra-annual as well as inter-annual spatio-temporal pattern
82 of migrations may vary due to e.g. changes in environmental conditions (Fréon and Misund, 1999;
83 Dingle & Drake, 2007), abundance (Fernö *et al*., 1998), fish age (Harden Jones, 1968; Fréon &
84 Misund, 1999), condition (Slotte, 1999) or geographic variation in food availability (Kvamme *et al*.,
85 2003).

86 The WBSS migration has been characterised as a summer feeding migration from spawning areas
87 distributed in fjords, sils and lagoons to the open waters of Kattegat and in particular the Skagerrak and
88 Eastern parts of the North Sea (Figure 1), followed by a return to wintering areas (Nielsen *et al*., 2001;
89 Payne *et al*., 2009). An age-related migration distance gradient has been reported for WBSS (Payne *et*
90 *al*., 2009) with the older individuals migrating furthest into the outermost area of the Skagerrak and
91 into the North Sea where the feeding conditions are supposed to be optimal (Maar *et al*., 2013).
92 However, given the several factors that have been shown to impact migration of *C.harengus*, the
93 variability in migration distance (from the main spawning ground in the Western Baltic to the optimal
94 feeding grounds in the outer Skagerrak, Figure 1) between years for WBSS could depend on other
95 factors than age. They e.g. may migrate further to get to the optimal feeding grounds when they are in

96 better than average condition. Alternatively, migration distance may be determined by local carrying
97 capacity, in which case the proportion migrating towards attractive areas is higher when abundance is
98 low. It has been shown for NSAS that the preferred spawning more Southern spawning grounds are
99 used independent of the stock size, while the less preferred more Northern grounds are used when the
100 stock size is increasing (Corten 2001). Thus abundance related habitat selection may be occurring in
101 *C.harengus* as it has been shown for other species (e.g. *Gadus morhua* in the North Sea (Blanchard *et*
102 *al*., 2005).

103 The present study examines the observed distribution, growth and condition of *C. harengus*
104 encountered in the mixed feeding aggregations in the Eastern North Sea, the Skagerrak and the
105 Kattegat during summer. From the distribution of NSAS and WBSS during six consecutive years of
106 acoustic surveys, the migration mechanisms most likely to determine the early summer distribution of
107 the age classes of the two major stocks were investigated. The analysis tested four hypotheses to
108 understand mechanisms and implications of stock mixture in the summer feeding area:

109 H1: Migration is predetermined by predictive orientation mechanism (genetic factors or imprinting)
110 towards predefined areas typically characterised by bottom topography and persistent hydrographical
111 features acting as an attractor. Thus the distribution of a population will appear constant, albeit with
112 random variation.

113 H2: Migration is directed towards the area showing optimal growth conditions.

114 H3: Migration is size dependent with larger fish migrating further than small. Thus the proportion of
115 individuals of WBSS in the North Sea will have a larger body size on average than the individuals
116 encountered in the Kattegat, closer to the main spawning site.

H4: Migration is a result of local carrying capacities. In this case, the abundance of NSAS and WBSS in the preferred area will seem constant independent of total *C.harengus* abundance while vary in other areas.

METHODS

SURVEY DATA

Hydro acoustic survey data on distribution, size and weight at age for *C. harengus* in the Kattegat and the Skagerrak was available for the years 2006 to 2011. The acoustic survey is part of the ICES Coordinated Acoustic Survey in the Skagerrak and Kattegat, the North Sea, West of Scotland and the Malin Shelf area (ICES, 2012).

Acoustic data were collected using a 38 kHz echosounder with the transducer mounted in a towed body towed at a target depth of 4-5 m depth. The raw acoustic data were pre-integrated into 1 m depth samples for each ping and combined into 1 nautical mile datasets. The acoustic data were scrutinized in depth layers for each nautical mile using special judging software which allows ignoring data from layers and/or intervals with noise. In areas with acoustic input from plankton and jellyfish, manually adjustable thresholds were applied to eliminate echoes from these objects. Final integration was conducted from 3 m below the transducer to 1 m above the bottom or to a maximum depth of 150 m. The area with depth above 150 m contributes to 31 % of the entire survey area. The integration yielded the total backscattering cross section, s_A, of fish per square nautical mile for each nautical mile along the survey track.

For each ICES statistical rectangle, a mean areal back scattering, s_A-value, was calculated based on the s_A-values for all sampled nautical mile inside the area. This value is assumed to represent the whole ICES statistical rectangle and is multiplied with its total area to obtain the total backscattering cross section of fish in the ICES statistical rectangle. Based on allocated trawl hauls in each ICES statistical rectangle or, if necessary, hauls from nearby ICES statistical rectangle, the species and length composition of fish were identified. The mean back scattering cross section, TS, for fish in the subarea was estimated based on the relative composition of fish in the mean catch and the length dependent TS-relationships of *C. harengus, S.spratus, Gadoid sp* and *S. scombrus* (ICES, 2012). The total number of fish in the subarea is then the total backscattering cross section of fish divided by the mean scattering cross section of fish. The number of fish per species is assumed to be proportional to the contribution of the given species in trawl hauls.

For each haul used for identification of species and length composition, the total catch was weighed, sorted into species and total weight and length distribution per species was recorded. Clupeids were measured to the nearest 0.5 cm total length below, and weighed to the nearest 0.1 g wet weight. In each trawl haul 10 (if available) *C. harengus* per 0.5 cm length class were sampled and frozen for individual laboratory determination of length, weight, age, and spawning type (NSAS or WBSS).

BIOLOGICAL PARAMETERS

In the laboratory, the length stratified subsamples of *C. harengus* were thawed and total length (nearest mm) and wet weight (0.01 gram) was recorded for each fish. The number of otolith winter rings (WR) was determined using the procedure described in ICES (2003) and entered as a proxy for age. The reason for using winter rings and not age in years is that the *C. harengus* are spawned either in autumn (NSAS) or spring (WBSS) and given that the NSAS only have approximately 3 months to live before

158 they experience their first winter, their first winter ring is not recordable and their first visible winter
159 ring is actually their second experienced winter (where they thus are 1.5 years old). The WBSS on the
160 other hand are less than 1 year old, when they lay down their first recordable winter ring. Otolith
161 microstructure (OM) was used to separate *C. harengus* stocks according to their different hatching time
162 using visual inspection of season-specific daily increment pattern in the larval otolith (Mosegaard &
163 Madsen 1996; Clausen *et al*., 2007b). The method discriminates between sympatric *C. harengus* with
164 different spawning times (Brophy & Danilowicz, 2002, 2003; Clausen *et al*., 2007b). Separation of
165 North Sea *C. harengus* from Western Baltic *C. harengus* in the Kattegat, the Skagerrak and the eastern
166 North Sea follows the assumption that all North Sea *C. harengus* are autumn/winter spawners and all
167 Western Baltic *C. harengus* are spring spawners as multiple populations with similar spawning time
168 cannot be distinguished with this analysis alone (Clausen *et al*., 2007). From 2010 onwards, harmonic
169 coefficients from Elliptic Fourier Analysis (EFA) of silhouette otolith images and non-parametric
170 nearest neighbour Discriminant Analysis (DA) were used together with OM to classify production
171 samples after calibration with an OM determined known-stock base-line (Burke *et al*., 2008). The OM
172 analysis is assumed to have less than 5% misclassification error of the base-line (Clausen *et al*., 2007b)
173 and cross-validated self-assignment shows about 10% misclassification of the EFA based DA of the
174 production samples (ICES, 2013a).

175 DISTRIBUTION

176 Changes in distribution over time were evaluated using two different methods. Firstly, the yearly
177 biomass across winter ring groups by ICES statistical rectangle for each area and spawner type was
178 investigated to determine whether a trend over time could be detected. Secondly, , two indicators of
179 distribution were estimated: The centre of gravity of location by stock and age and changes in the area

covered were investigated by estimating the average squared distance. The former indicator reflects whether the distributional area has changed geographical location whereas the latter indicator reflects changes in the area covered by the stock. Centre of gravity was calculated by the mid-point latitude and longitude in each ICES statistical rectangle weighted by the biomass of age categories in each ICES statistical rectangle:

$$\left(C_{lon,s,y,t}, C_{lat,s,y,t}\right) = \left(\left(\sum_{i=0}^{N} B_{s,y,t,i} lon_i \right) \left(\sum_{i=0}^{N} B_{s,y,t,i} \right)^{-1}, \left(\sum_{i=0}^{N} B_{s,y,t,i} lat_i \right) \left(\sum_{i=0}^{N} B_{s,y,t,i} \right)^{-1} \right)$$

Where $C_{lat,s,y,t}$ and $C_{lon,s,y,t}$ is the latitude and longitude of the center of gravity of spawner type *s* with *t* winter rings in year *y*, $B_{s,y,t,i}$ is the biomass of spawner type *s* with *t* winter rings in year *y* in the *i*th rectangle and lat_i and lon_i is the mid latitude and longitude of ICES rectangle *i*, respectively.

Changes in the area covered were investigated by estimating the average squared distance, *D*, of a biomass unit to the centre of gravity:

$$D_{s,y,t} = \left(\sum_{i=0}^{N} B_{s,y,t,i} \left(\left(lat_i - C_{lat,s,y,t} \right)^2 + \left(lon_i - C_{lon,s,y,t} \right)^2 \right) \right) \left(\sum_{i=0}^{N} B_{s,y,t,i} \right)^{-1}$$

This index is proportional to the area covered by 95% of the biomass if the distribution is a two-dimensional normal distribution in space and even when the distribution is skewed or in other ways deviate from normality, this indicator still reflects concentration of the stock (Rindorf & Lewy, 2012). The indicator is not responsive when the distribution is bimodal, but judging from the distribution, this was not a problem in our analyses.

Age related changes in the combined effects of mortality and migration were investigated by general linear models of log(numbers) at age by area and type to compare slopes of the observed decline in numbers with expected total mortality to infer immigration and emigration patterns among areas. The difference in slope between cohorts, years and areas were also investigated to determine whether different cohorts experienced differences in mortality. Further, the presence of higher declines for older ages, corresponding to higher mortality or emigration, was tested by estimating a second degree polynomial relationship between age and log(numbers at age).

GROWTH BY AREA

The difference in length at age between area and spawner type was investigated to determine which of the areas could be considered most favourable for growth or alternatively attract a specific growth type. This was done through comparing length at age in a specific area with length at other ages and estimating a von Bertalanffy growth equation across all years. This method will provide a combined estimate of the effect of growth, size selective mortality and size specific net migration in an area. The analysis is referred to here as an analysis of growth, which pertains to the assumption that size specific net migration are of minor importance in comparison to growth in our results. This assumption is discussed when interpreting the relationship between apparent growth and changes in distribution.

The relationship between area and spawner type and length at age was estimated through analyses of the parameters of the von Bertalanffy growth equation using data from all years ignoring any cohort or year effects. Von Bertalanffy growth equation for all areas and types was estimated as follows:

$$L_{a,s,t,i} = L_{\infty,a,s}\left(1 - \exp\left(-K_{a,s}(t - t_{0,a,s})\right)\right) + \varepsilon_i$$

Where *t* denotes number of winter rings, $L_{a,s,t,i}$ is the average total length in the *i*th observation (ICES rectangle) at *t* winter rings in area *a* and spawner type *s*, $L_{\infty,a,s}$ is the average length of very old *C. harengus* in area *a* and spawner type *s*, $K_{a,s}$ is the growth rate in area *a* and spawner type *s*, $t_{0,a,s}$ is the theoretical age at which length is zero in area *a* and spawner type *s* and ε_i is an error term, $\varepsilon_i \in N(0,\sigma_\varepsilon)$. Parameters were estimated by least squares and recorded lengths at age 0 were excluded from analyses. The effect of the factors area *a* and spawner type *s* on the parameters was evaluated using an F-test and a significance level of 0.01. This lower significance level was chosen to accommodate the fact that the observations were not strictly independent (within-year correlation, see results) and to avoid including factors which, though significant, explain a very low amount of the variation. The length anomaly of the individual observation was defined as the residual length from the estimated von Bertalanffy relationship and was calculated and used for further analyses of yearly differences. Length-anomalies were investigated for trends by estimating the parameters in a generalized linear model, investigating the effects of type and year for each area separately assuming a normal distribution of anomalies.

CONDITION

An average condition index was calculated for each type, year, statistical rectangle and age by first estimating the common weight-length relationship

$$W = aL^b$$

for all observations using a generalized linear model with gamma distributed error in mean weight to estimate *b*. The average condition, C_i of a given combination of type, year, statistical rectangle and age (observation *i*) was then estimated as

$$C_i = W_i L_i^{-b}$$

The difference in condition between spawner types, years and areas was investigated using ANOVA whilst the trend over time in a generalized linear model with year as a linear variable, and the effect of length on condition by area was tested between immature and mature *C. harengus* of both spawner types using the same method.

RESULTS

DISTRIBUTION

C. harengus distribution at different scales was variable among years (Figure 2) as was the annual proportion of spawner types by square. Analysis of distribution by year shows that the total distribution and relative abundance shifts between years based on data collected during the summer acoustic cruise (Figure 2), thus *C.harengus* do not necessarily congregate in the same area each summer. Total biomass of spring spawners has been decreasing over the period in Kattegat (correlation between year and biomass per rectangle=-0.58, $P<0.0001$) and Skagerrak (correlation between year and biomass per rectangle=-0.30, $P=0.0094$) (Figure 3). In contrast, there was no significant trend in the biomass of spring spawners in the North Sea or in biomass of autumn spawners in any area ($P>0.20$ in all cases, Figure 3). The decline in total biomass of spring spawners over the time period was 81.4% in

253 Skagerrak and 95.9% in Kattegat. As the biomass declined over time, the proportion of the total
254 biomass for the area which constituted spring spawners decreased accordingly. This decrease was
255 significant in both the North Sea (P=0.0396) and Kattegat (P=0.0080), but was below the significance
256 level in Skagerrak (P=0.0759).

257

258 The shift in distribution is also seen when examining the centre of gravity of the two spawner types
259 (Figure 4); there is no change in either latitude or longitude of the centre of gravity of autumn spawners
260 (P>0.50 in both cases). On the other hand, the longitude of the centre of gravity decreased significantly
261 for the spring spawners in the period corresponding to a westward shift in distribution (correlation=-
262 0.40, P=0.0147) whereas there was no significant change in latitude of center of gravity (P>0.23). The
263 centres of gravity by age class were significantly positively correlated along the latitudinal component
264 (r=0.61, P=0.0002), primarily driven by an age class related tendency of old *C. harengus* to be located
265 in more Northern (deeper waters) in Skagerrak and the North Sea (Figure 4). No such correlation
266 existed along the longitudinal dimension, but spring spawners had a significantly more easterly
267 distribution than autumn spawners for all ages (P<0.0001). The distribution coverage (*D*) of the two
268 stocks showed year effects but no trend and coverage was not significantly correlated to biomass
269 (P>0.16 in all cases).

270 Abundances expressed as log transformed numbers declined linearly with increasing age as expected
271 (Figure 5). Slopes for autumn spawners exhibited no area effects (P=0.1410), corresponding to a
272 similar combined effect of mortality and migration in all areas, whereas a significant area effect on
273 slopes for spring spawners was found (P<0.0001). Slopes were significantly non-linear for autumn

spawners (P<0.0001), whereas no significant non-linearity was found for spring spawners (P=0.4595). Slopes for autumn spawners were overall steeper (slope=-1.37 (se=0.09)) than for spring spawners and substantially higher than the estimated F (fishing mortality) + M (natural mortality) (0.66) in stock assessment would suggest (ICES, 2013b) indicating either a higher mortality or an emigration of autumn spawners. Spring spawners in the Skagerrak and the North Sea exhibited declines which were lower than those expected from stock assessment estimates of total mortality (0.39 (se=0.11) and 0.58 (se=0.07), in the North Sea and Skagerrak, respectively), whereas spring spawners in the Kattegat had a significantly higher negative slope (1.14 (se=0.09)) indicating either emigration from Kattegat to Skagerrak of the North Sea or substantial differences in mortality between areas.

Abundance fluctuations were similar within each area for *C. harengus* age-classes more than 1 wr, whereas the youngest age class to a lesser degree followed fluctuations of older *C. harengus*. Fluctuations in the Kattegat and the Skagerrak exhibited apparent inverse patterns (whereas the Eastern North Sea was uncorrelated to the other areas and more stable (Figure 5)). Autumn spawner fluctuations were positively correlated in the Skagerrak and the Eastern North Sea but not in other combinations.

Although spring spawners in the North Sea had a single low abundance outlier in age group one (in 2007), analysis of residual ln(N) pattern did not show significant underrepresentation of age 1 spring spawners in any of the areas (P>0.7 for all three areas), indicating that migration to all feeding areas generally takes place already at 0-1 wr. ln(N_1) of autumn spawners was significantly over represented

294 in the Skagerrak (P=0.03), indicating that the high emigration rate of autumn spawners starts already at
295 age 1-2 wr.

296 COMPARISON OF DISTRIBUTION, GROWTH AND SIZE AT AGE

297 Von Bertalanffy K and t_0 did not differ significantly between spawner types or between areas (P>0.01
298 in all cases), whereas L_∞ differed both between spawner types and areas (P<0.0001 in both cases). This
299 indicates that length at age is similar for the youngest ages but becomes increasingly different between
300 types and areas with age (Figure 6). The variation in L_∞ between areas explained 49% of the residual
301 variation in mean length around a common von Bertalanffy relationship, and variation in L_∞ between
302 spawner types another 11%, leading to a total of 60% of the residual variation explained by the final
303 model. The estimated L_∞ in the North Sea did not differ significantly from that in the Skagerrak
304 (P=0.20). However, to avoid introducing a growth period related bias in the subsequent analyses (see
305 methods), separate estimates were derived from the two areas. The resulting parameter estimates can be
306 seen in Table 1. The variables L_∞, K and t_0 were highly correlated (all correlations>0.75) as is generally
307 the case when estimating von Bertalanffy parameters.

308 The growth anomalies (the residuals from the reduced von Bertalanffy model) did not differ
309 significantly between spawner types in any of the areas (P>0.09 in all areas, Figure 7) and there was no
310 significant differences between years in the North Sea (P=0.8838). However, the residuals varied
311 significantly between years in the Skagerrak and Kattegat (P=0.0030 and P=0.0003, respectively). The
312 year effect in residuals violates the assumption of independent residuals and hence the degrees of
313 freedom used when reducing the von Bertalanffy model are likely to be overestimated and parameter
314 error estimates are likely to be minimum estimates. In the Skagerrak, the differences did not result in a

315 trend over time (P>0.20), whereas the difference between years in Kattegat introduced a significant
316 negative trend in residuals (P<0.0001) with the average residual decreasing by 0.38 cm per year.
317 Hence, the Kattegat fish not only had the lowest L_∞ and hence the lowest size at age at all ages, this
318 measure also declined progressively over the time period.

319 CONDITION

320 Condition differed significantly between spawner types (P<0.0001) with condition being $0.12*10^{-3}$
321 $g*cm^{-3.26}$ (std=$0.02*10^{-3}$ $g*cm^{-3.26}$) higher in autumn spawners than spring spawners. The difference
322 between areas was also highly significant (P<0.0001), with both types showing the highest condition in
323 the North Sea and the Skagerrak while condition was substantially lower in the Kattegat (Table 2). A
324 significant correlation was found between condition of the two spawner types by ICES rectangle year
325 and age within all age groups with age 1 having the highest correlation r=0.81 p<0.0001, and in all
326 cases r>0.4, P<0.05 (Figure 8).

327 Condition decreased significantly with age ($-0.070*10^{-3}$ $g*cm^{-3.26}$ per year, standard error=$0.007*10^{-3}$
328 $g*cm^{-3.26}$) with no significant difference in the decrease between areas (P=0.3809) or spawner types
329 (P=0.6801). No significant correlation between condition by age class and biomass per ICES rectangle
330 was found for any of the two spawner types, indicating local density independence of condition.

331 For both spring and autumn spawners condition decreased significantly with age. In addition to a
332 significant area effect (P<0.0001), spring spawners exhibited a significantly different relationship with
333 residual length for juvenile and adult spring spawners over all areas, showing a non-significant
334 negative slope for 1-2 wr and a significant positive slope for 3-5 wr (slopes -0.06, R^2=0.06 and 0.21,

335 R^2=0.33 respectively, P=0.003 for slopes being equal). No trends in condition with growth rate and no
336 significant differences between juveniles and adults were found in autumn spawners (P=0.12).

337 # DISCUSSION

338 This study showed significant variation in the distribution of western Baltic spring spawners and North
339 Sea autumn spawners in their summer feeding area, rejecting the hypothesis that the summer feeding
340 migration of these two stocks in the study area is predetermined by predictive orientation mechanism
341 (H1). *C. harengus* in the Skagerrak and the Eastern North Sea were in general significantly larger than
342 in the Kattegat and the former areas exhibited consistently higher abundance than Kattegat. Spring
343 spawners migrated to the Skagerrak and the North Sea from 1wr whereas autumn spawners appeared in
344 all three areas from the earliest age but started to leave all areas at least between 1 and 2 wr. Size at
345 age did not differ between areas at 0 wr, but differences emerged with increasing age, supporting the
346 conclusion of differences in growth rate while indicating that migration was at least not initially size
347 dependent. Thus the migration appeared to be size dependent directed towards the area showing
348 optimal growth conditions, confirming hypotheses H2 and H3 of this study. Density in the low growth
349 area Kattegat decreased substantially faster than could be explained by the expected mortality levels,
350 corresponding with a density dependent migration towards areas where growth rate appears to occur at
351 a faster rate or an increased emigration as growth conditions deteriorated. This indicates that the
352 summer feeding migration is a result of local carrying capacities given that the abundance of NSAS and
353 WBSS in the preferred area was independent of total *C.harengus* abundance while it significantly
354 decreased with total abundance in Kattegat; thus confirming hypothesis H4 of the study.

Estimated biomass for spring spawners declined substantially in both the Kattegat and the Skagerrak over the period, whereas no trends were found for autumn spawner biomasses in any of the three areas. The cause of the decline was likely a combination of high fishing pressure and decreasing recruitment during the first decade of the 2000s (ICES, 2013b). The spring spawners apparently kept migrating as far as the North Sea at the same time as they became fewer and smaller at age in the Kattegat, thus the remaining part of the stock seemed to prefer feeding areas further from the spawning grounds regardless of initial size**.** Areal coverage of the spring spawning stock did not co-vary with decreasing biomasses and thus did not follow the hypothesis of contracting feeding range with declining population size (Murphy, 1977).

The North Sea and Skagerrak parts of the summer-feeding area were at a constant advantage in terms of the largest size at age and the highest condition across years, independent of spawning type. The difference in L_∞ between the North Sea-Skagerrak and the Kattegat was around 3 cm for both spawning types and even in years with positive size at age anomaly in the Kattegat, they still exhibited the smallest size as the anomaly never exceeded 1 cm (Table 1, figure 7). Thus, the Eastern North Sea-Skagerrak likely provided the best growth opportunities for *C. harengus* irrespective of spawning type and year. *C. harengus* is known to be a size selective planktivore, preferring large-sized e.g. calanoid zooplankton species, as seen in the Baltic (Flinkman *et al*., 1998), the North Sea (Maravelias *et al*., 2000; Last, 1989; Segers *et al*., 2007) the Norwegian Sea and the North Atlantic (Dalpadado *et al*., 2000; Gislason & Astthorsson, 2002) and the Gulf of St. Lawrence (Darbyson *et al*., 2003). The available literature and data on the zooplankton community in the Kattegat-Skagerrak area suggest that higher concentrations of egg producing adult stages of the *Calanus finmarchicus* (Gunnerus) follow frontal zones coupled to the Skagerrak loop of Jutland current and low saline waters entering the

377 Skagerrak from the Kattegat (Maar *et al*., 2013). Also, the community of larger zooplankton changes in
378 the transition zone between the Baltic and the North Sea; euphausiids increase significantly in size
379 from Kattegat to Skagerrak (Buchholz & Boysen-Ennen, 1988). Thus supremacy in food quality and
380 availability in the Eastern North Sea-Skagerrak may explain at least some of the difference observed
381 here in growth pattern between areas.

382 *C. harengus* biomass dominates the pelagic fish community in the Skagerrak and surrounding areas,
383 but *C. harengus* condition and apparent growth rate exhibited divergent co-variation with *C. harengus*
384 abundance in the three sub-areas. There was an increase in condition in the North Sea and Skagerrak
385 concurrent with the decrease in biomass in the Skagerrak, whereas both condition and size at age of
386 spring spawners in Kattegat decreased over the time-period concurrently with a marked decrease in
387 biomass. Evidence of density dependent growth has been found for several stocks of Atlantic *C.*
388 *harengus* (Icelandic summer spawners (Oskarsson, 2008), Norwegian spring-spawners (especially for
389 immature fish; Toresen, 1990), Georges Bank (Melvin & Stephenson, 2007), and Baltic Sea (Casini *et*
390 *al*., 2006), but not for others (Gulf of Finland, and southern Gulf of St Lawrence, as reviewed in
391 Melvin & Stephenson (2007)). *C. harengus* in the Eastern North Sea and Skagerrak did not display any
392 trend in growth rate over time. In contrast, the condition of *C. harengus* in the Kattegat was
393 consistently poorer than that of *C. harengus* in the Skagerrak and the Eastern North Sea across years
394 and spawning type, which supports the conclusion that Kattegat is less optimal for summer growth. The
395 opposing trends in condition in Kattegat and Skagerrak concurrent with the order of magnitude
396 decrease in the Kattegat and Skagerrak biomasses also indicates that the decrease in size at age in the
397 south-eastern part of the summer distribution area is unrelated to density dependence in the two stock
398 sub units.

399 A decrease in length at age also acts to decrease biomass. However, the decrease in asymptotic length
400 in Skagerrak and Kattegat was 1.7 cm and 2.2 cm, respectively, corresponding to about 18% and 25 %
401 decrease in individual weight, which is clearly insufficient to explain the 81% and 96% decrease in
402 biomass in the Skagerrak and the Kattegat, over the entire time period. The latest stock assessment
403 estimates of the total Western Baltic Spring Spawner total biomass shows a decline of 47% from 2006
404 to 2011, whereas North Sea Autumn Spawner total biomass has increased by about 17% (ICES,
405 2013b). A marked difference in size selectivity and intensity of both fishery and natural predators in
406 Kattegat compared to Eastern North Sea and Skagerrak could also explain the change in biomass and
407 different growth rate pattern. However, since 2002, the *C. harengus* fishery in the area has been
408 concentrated in the more north-western part of Skagerrak (ICES, 2013b). Although the predation field
409 may differ between the areas, it seems unlikely that predators should be responsible for an increasing
410 outtake of larger *C. harengus* in the Kattegat only and hence be the cause of the decreasing length at
411 age.

412 A significant year effect on residual length in both the Kattegat and the Skagerrak is only matched by a
413 similar cohort effect in the Skagerrak, indicating that the stock components in the Kattegat are not
414 persistent among years and they most likely redistribute to the Skagerrak at older ages This is further
415 supported by the much steeper slope of the log transformed cohort numbers for both spawning types in
416 the Kattegat. The variation in annual center of gravity for both stocks in the area is much greater than
417 for the autumn spawners' center of gravity in the North Sea during the same period and time of the year
418 (ICES, 2013b). The lack of correlation between biomass and distributional trends in the transition area
419 indicates that the search for the best feeding opportunities shifts the population distribution annually.
420 This is overlaid by a westward migration tendency of the autumn spawners and a gradual shift towards

421 deeper waters with increasing size for both spawning types. Hence, changes in biomass levels and
422 centres of gravity as well as patterns in size at age all point to a redistribution of *C. harengus* towards
423 more north-western parts of the summer feeding area during a period when the spring spawner
424 population declined. The observed population mobility among years indicates that local changes in
425 environmental conditions may be the drivers behind the general distribution pattern.

426 Sudden density dependent changes in growth rate are not uncommon in *C. harengus* and may appear as
427 a regime shift mediated through interspecific clupeid competition as in the Baltic (Möllmann *et al*.,
428 2005) or intraspecific competition in the Gulf of Riga (Raid *et al*., 2010) where an increase in *C.*
429 *harengus* abundance in the late 1980s changed growth conditions to much smaller maximum size at
430 age. Further, a large *C. harengus* year-class may suppress the individual growth in the cohort and exist
431 as a marker for the entire life span as seen for the 1904 year-class of Norwegian spring spawners (Hjort
432 1914) and the 2000 year-class of the North Sea autumn spawners (ICES, 2013b).

433 The two *C. harengus* stocks in the area exhibit marked differences in their innate migration behaviour
434 that probably reflect stock-specific differences in spawning time and location. Autumn and winter
435 spawned *C. harengus* larvae drift during winter from the western and southern parts of the North Sea
436 towards their later nursery areas including the transition area of the eastern North Sea, the Skagerrak
437 and the Kattegat (Johannesen & Moksness 1988). Larvae from the spring spawning stock are dispersed
438 locally (Polte *et al*., 2013) and juveniles therefore actively have to migrate to the nursery grounds in the
439 transition area.

440 The proportion of spring spawners increases from age 1 to age 2 wr in the Eastern North Sea part of the
441 summer feeding area (ICES, 2013b), and it has generally been assumed that the full migration distance

of the spring spawning stock is first attained at the age 2 wr (Payne *et al*., 2009). However, our analysis of age class decline as slope of log abundance indicates that in five out of six years, age 1 spring spawners migrate as far towards the North Sea as their older relatives. The higher slope in the corresponding analysis of autumn spawners indicate that juveniles of this stock leave the nursery area in high numbers already at 1 wr, leading to an increase in spring spawner proportion in the transition area from age 1 to age 2 .

During the growing season, juvenile *C. harengus* join schools of similar sized individuals (Nøttestad *et al*., 1999). The findings here indicate that initially juveniles from both stocks form mixed schools in the area and gradually relocate according to experienced growth potential. A higher occurrence of mixed juvenile schools in the summer feeding area early in life when the two *C. harengus* types are of the same size would explain the higher correlation in condition between spring and autumn spawners at 1 wr. Further the lack of positive correlation between condition and residual size in juvenile spring spawners as opposed to adult spring spawners could be explained if spring spawner juveniles with the highest growth potential would school with the largest and fastest swimming autumn spawner juveniles.

The apparent advantage in terms of growth rate associated with the western parts of the summer distribution area would mean that to optimize growth, individual *C. harengus* should spend the summer feeding period there. As the difference in size at age in the two areas in terms of both length and condition increased over time, the distribution concurrently shifted towards the high growth areas (Figure 7). Given that the autumn spawners did not systematically shift distribution over time, the distribution of the *C. harengus* biomass did not simply follow a given distribution of food items. Thus

463 for the spring spawners, the determining factor for the amplitude and direction of the summer feeding
464 migration is likely to differ from the determinant of the autumn spawner distribution.

465 The summer feeding migration pattern observed in the spring spawners appears to be consistent with
466 maximization of growth rate in the individual *C. harengus* where *C. harengus* with increasing age and
467 size progressively abandon the sub optimal feeding areas in the Kattegat to concentrate further to the
468 north-west. State dependent migration is a well-known behaviour in fish (Harden Jones, 1968) and for
469 *C. harengus* it has been well documented for Norwegian Spring Spawning *C. harengus* both
470 concerning spawning migration (Slotte & Fiksen, 2000) and summer feeding migrations (Kvamme *et*
471 *al*., 2003). In our study, the extent of the migration is probably defined at an early stage since the
472 differences in growth rates in the areas emerge with age (Figure 6). Thus the advantage in terms of
473 growth rate continues through life for the individuals reaching furthest in the migration. However,
474 given the westerly change in distribution over time (Figure 4) during the years where the growth
475 conditions in Kattegat continues to worsen, indicates that this pattern can change and *C. harengus* can
476 benefit from improved opportunities for growth by changing their migration pattern, just as seen in
477 Norwegian Spring Spawners (Kvamme *et al*., 2003). The observed westward changes in distribution of
478 the spring spawning *C. harengus* may be caused either by increased mortality of fish in Kattegat, by a
479 general decrease in the stock combined with an increase in the migration distance of the average fish or
480 by a combination of the two. An increase in migration could be induced by generally increased size at
481 age or by the diminishing density dependent competition for resources in Skagerrak/North Sea as
482 density decreases. The former seems unlikely as length at age residuals decreased in both Skagerrak
483 and Kattegat, indicating that the fish did not need to have a threshold condition/size to move to
484 Skagerrak. In that case, size at age would have remained unchanged in Skagerrak. There could be

485 indications of a threshold size to move to the North Sea as no change in residual length was seen here.
486 If density dependent competition for resources has limited migration of smaller fish so far, this effect
487 should diminish in Skagerrak in later years, given the reduction in biomass recorded, leaving room for
488 more fish to move to this area. If these fish were among the larger fish in Kattegat, this movement
489 would act to decrease length at age in both Skagerrak (now receiving smaller fish) and Kattegat (now
490 losing larger fish). If the effect is furthermore the result of accumulating effects on length at age at
491 different ages, this could explain why biomass in an individual year in spite of underlying density
492 dependent effect was not significantly related to residual length. Thus, a degree of size dependence of
493 migration distance may still exist though this does not show up in the current investigation.

494 The advantage in terms of growth rate in western areas and the westward displacement of the
495 distribution of spring spawners over time suggests that both growth rate and density of conspecifics
496 may influence the migration pattern of WBSS *C. harengus*. In a trade-off between migration
497 expenditure and energy accumulation for growth and later reproduction local *C. harengus* with a low
498 growth potential (expressed as a lower condition in all years) will not experience a net energy gain by
499 increasing migration distance and moving further out than Kattegat, displaying the same differences in
500 trade-off between migration length and spawning success as observed in migrating and non migrating
501 *C. harengus* in a Norwegian fjord (Johannesen *et al*., 2009).

502 This study demonstrates a growth related migration of both spring and autumn spawners directed
503 towards the more western parts of the summer feeding area, where the growth conditions are optimal.
504 This is a change in the perception of the mixture of *C. harengus* during summer in the area and it will
505 have consequences for the management of the fishery on these stocks during summer. The fishery in
506 the area takes mixed catches of juveniles from the two stocks whereas adult *C. harengus* in the catches

predominantly consist of spring spawners. The results of this study imply that catches of *C. harengus* in these areas with optimal growth conditions will consist of a faster-growing part of the stocks, which should be considered by the management of the *C. harengus* fishery. A mixed fishery targeting specific parts of a stock may lead to a reduction in the capacity of the stock to withstand climate variability and change; i.e. the resilience of the stock (Schindler et al., 2010). The distribution of the *C. harengus* in the area is thus more influenced by growth of the individual fish than the age of the fish. The change from the earlier perception of a limited age 1 migration (see Payne *et al*., 2009) to a full dispersion of all juvenile spring spawners to the entire summer feeding area, combined with the finding of progressive juvenile autumn spawner emigration will lead to different mixing of the stocks in juvenile fishery than previously assumed. This will influence the current procedure of predicting catch options to be considered in the management of the *C. harengus* by-catch in the small meshed sprat fishery in Division IIIa (ICES 2013a) given the need for considering the varying mixture of juvenile *C. harengus*. This study, thus, supports the notion (e.g. Schindler *et al*., 2010; Bekkevold *et al*., 2011) that marine fish management needs to incorporate knowledge about individual population dynamics to allow sustainable exploitation of all substocks.

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732

733 TABLE 1. Parameter estimates for the reduced von Bertalanffy model. Values in parentheses denote

734 95% confidence intervals.

Parameter	Area	Estimate
K	All	0.380 (0.279, 0.481)
t_0	All	-1.94 (-2.57, -1.31)
$L_{\infty,autumn}$	North Sea	29.7 (28.3, 31.0)
$L_{\infty,autumn}$	Skagerrak	29.4 (28.1, 30.6)
$L_{\infty,autumn}$	Kattegat	26.5 (25.2, 27.7)
$L_{\infty,spring}$	North Sea	28.6 (27.4, 29.9)
$L_{\infty,spring}$	Skagerrak	28.3 (27.1, 29.5)
$L_{\infty,spring}$	Kattegat	25.4 (24.3, 26.5)

744

745 TABLE 2. Average condition of autumn and spring spawners by area. Values in parentheses denote

746 standard error of the estimate. Units are 10^{-3}g*$cm^{-3.26}$

Area	Autumn spawners	Spring spawners
North Sea	3.70 (0.03)	3.58 (0.03)
Skagerrak	3.73 (0.02)	3.52 (0.02)
Kattegat	3.45 (0.04)	3.19 (0.02)

747

748 Figure 1. The study area. Straight lines indicate ICES management subdivision areas, shading indicate
749 spawning grounds of the WBSS stock based on literature (Biester, 1979; Otterlind, 1987; Rosenberg
750 and Palmén, 2982) and information from local fishermen. The circle represents the main spawning
751 ground (Greifswalder Bodden; Biester, 1979)

752 Figure 2. The weight proportion of spring spawners by year and statistical rectangle (grayscale colours)
753 as well as the relative total biomass by year and statistical rectangle (bubbles, areas are proportional to
754 total biomass of both populations but rescaled for each year; hence only within year comparisons are
755 possible).

756 Figure 3. Biomass of autumn (top) and spring (bottom) spawners per rectangle across years in the
757 North Sea (black symbols, black line), Skagerrak (grey symbols, grey line) and Kattegat (open
758 symbols, broken line). Lines are regression lines.

759 Figure 4. Left: annual centre of gravity for the autumn spawners (circles with grey dotted lines) and
760 spring spawners (circles med black solid lines) size and numbers within circles indicate year as in
761 20xx. Right: average centre of gravity for age classes 1-5 wr, autumn spawners (circles with grey
762 dotted lines) and spring spawners (circles med black solid lines) size and numbers within circles
763 indicate age (wr).

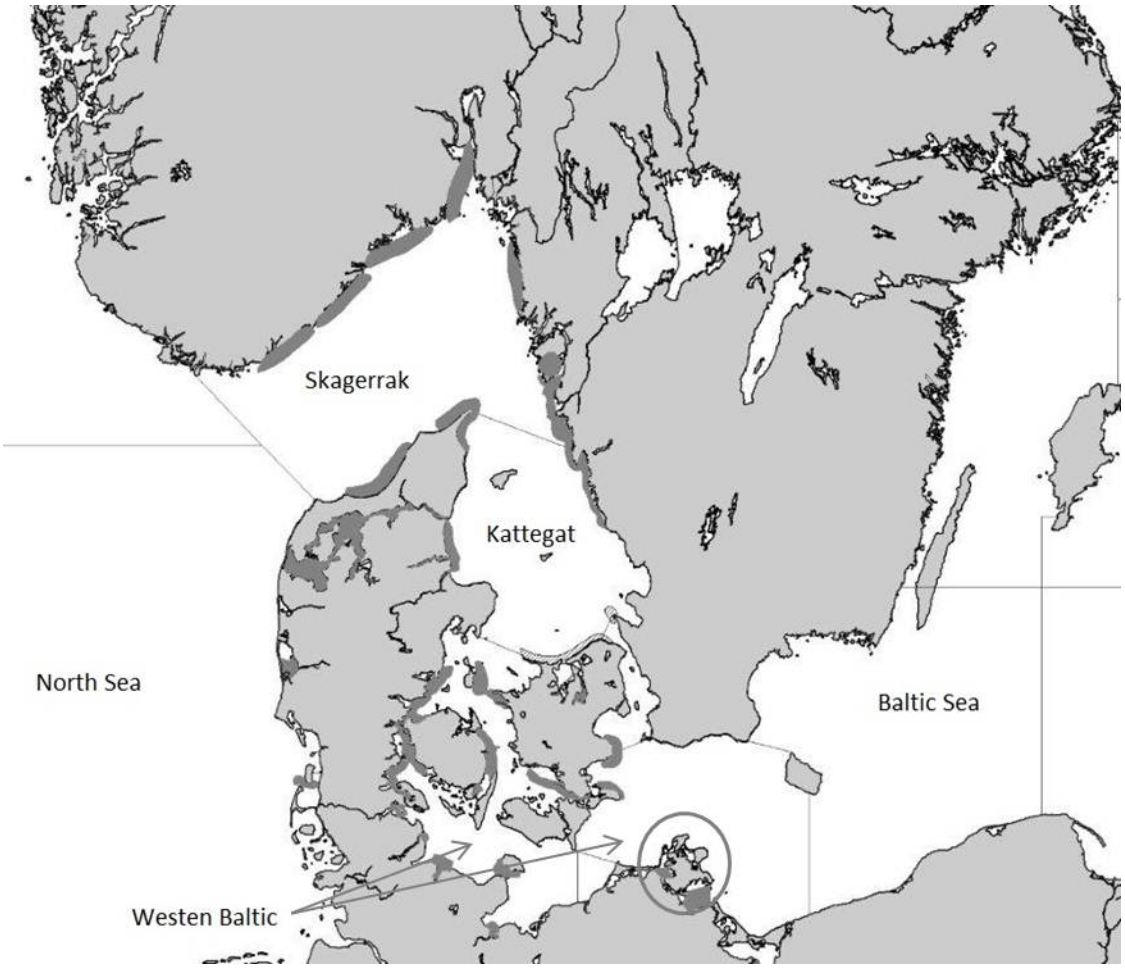
764 Figure 5. Ln(Catch in numbers) at age of autumn (left) and spring (right) spawners in the North Sea
765 (solid triangles, solid line), Skagerrak (open diamonds, dotted line) and Kattegat (open squares, dash
766 line). Lines are regression lines.

767 Figure 6. Predicted length at age for each area and type from the reduced von Bertalanffy model. Left:
768 Autumn spawners, Right: spring spawners. Black: North Sea and Skagerrak, grey: Kattegat. Solid line
769 denotes predicted length, hatched lines the 95% confidence interval around the prediction.

770 Figure 7. Residuals from the final von Bertalaffy model by year (growth anomalies). Autum spawners
771 (open symbols, hatched line) and spring spawners (closed symbols, solid line) in the North Sea (top
772 left), Skagerrak (top right) and Kattegat (bottom left).

773 Figure 8: Condition (C) of spring spawners vs condition of autumn spawners, by ICES rectangle, year
774 and age. Increasing size of bubbles indicates increasing age from 1 wr to 5 wr.

775



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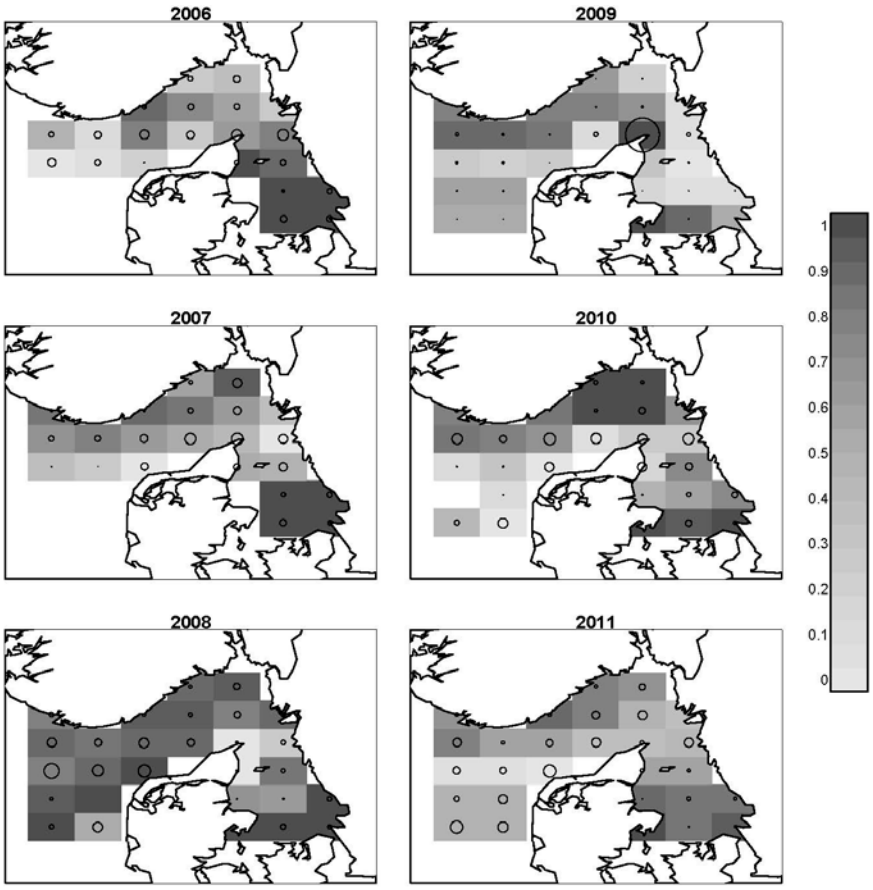

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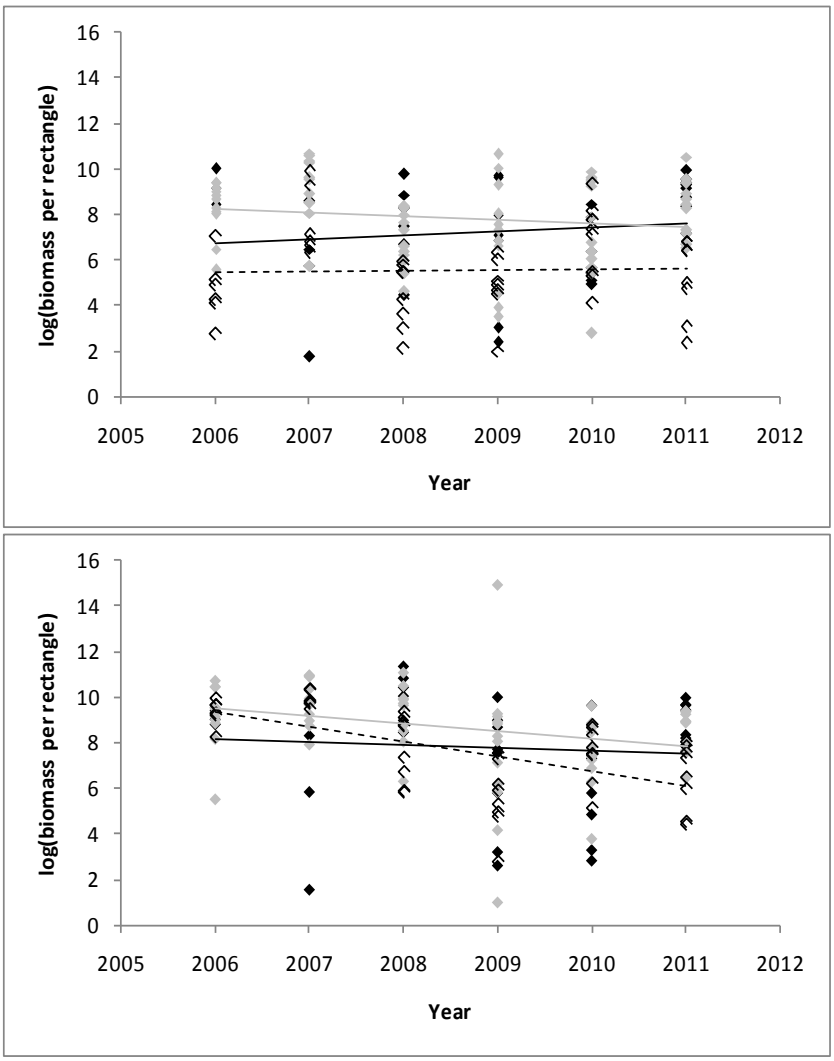

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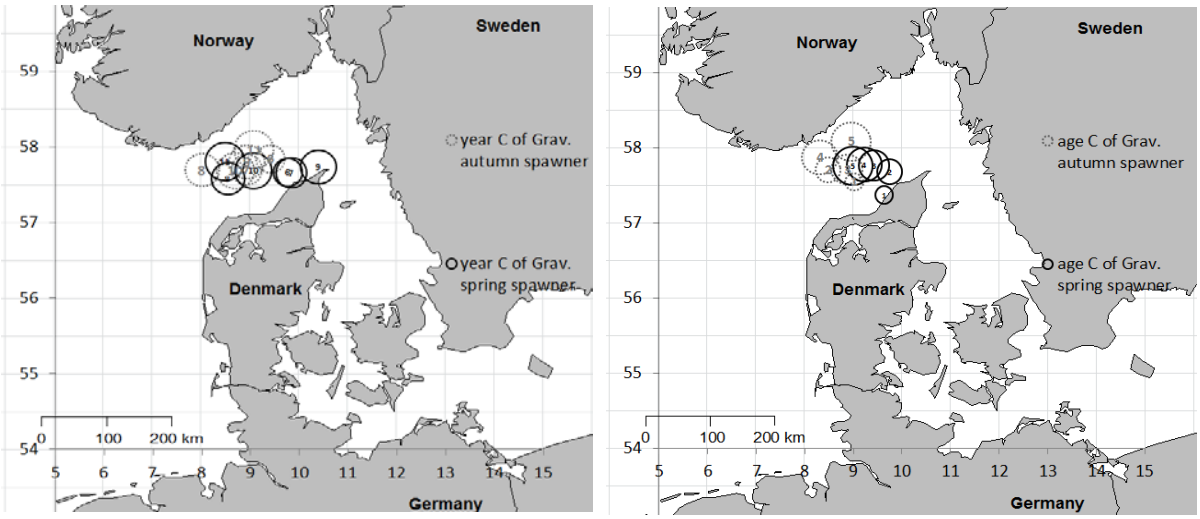

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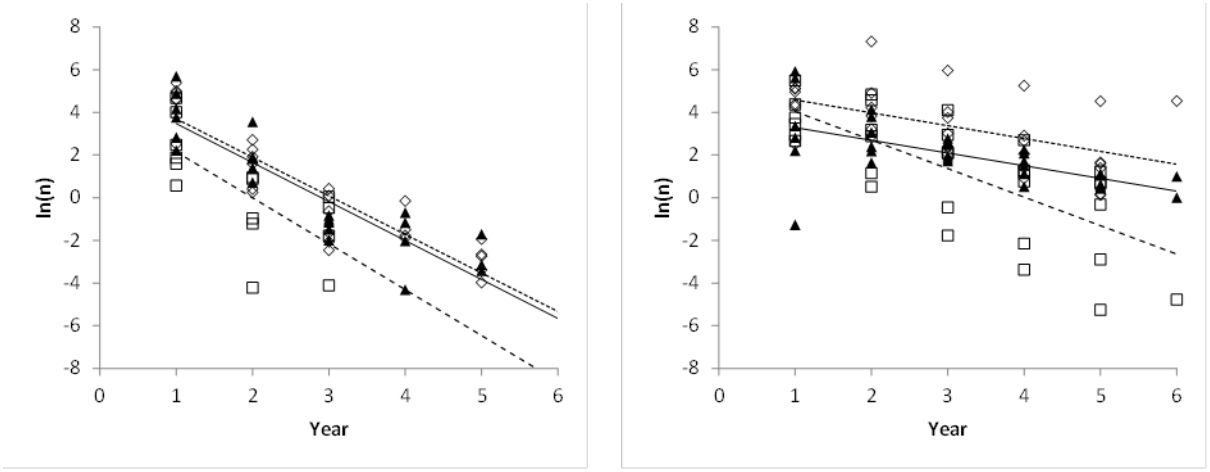
784 Figure 4.



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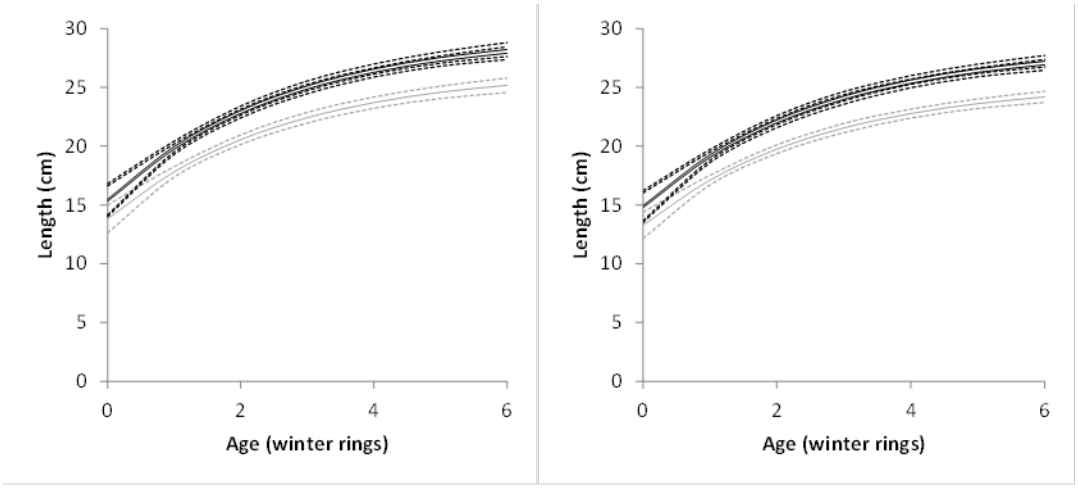
786 Figure 5.



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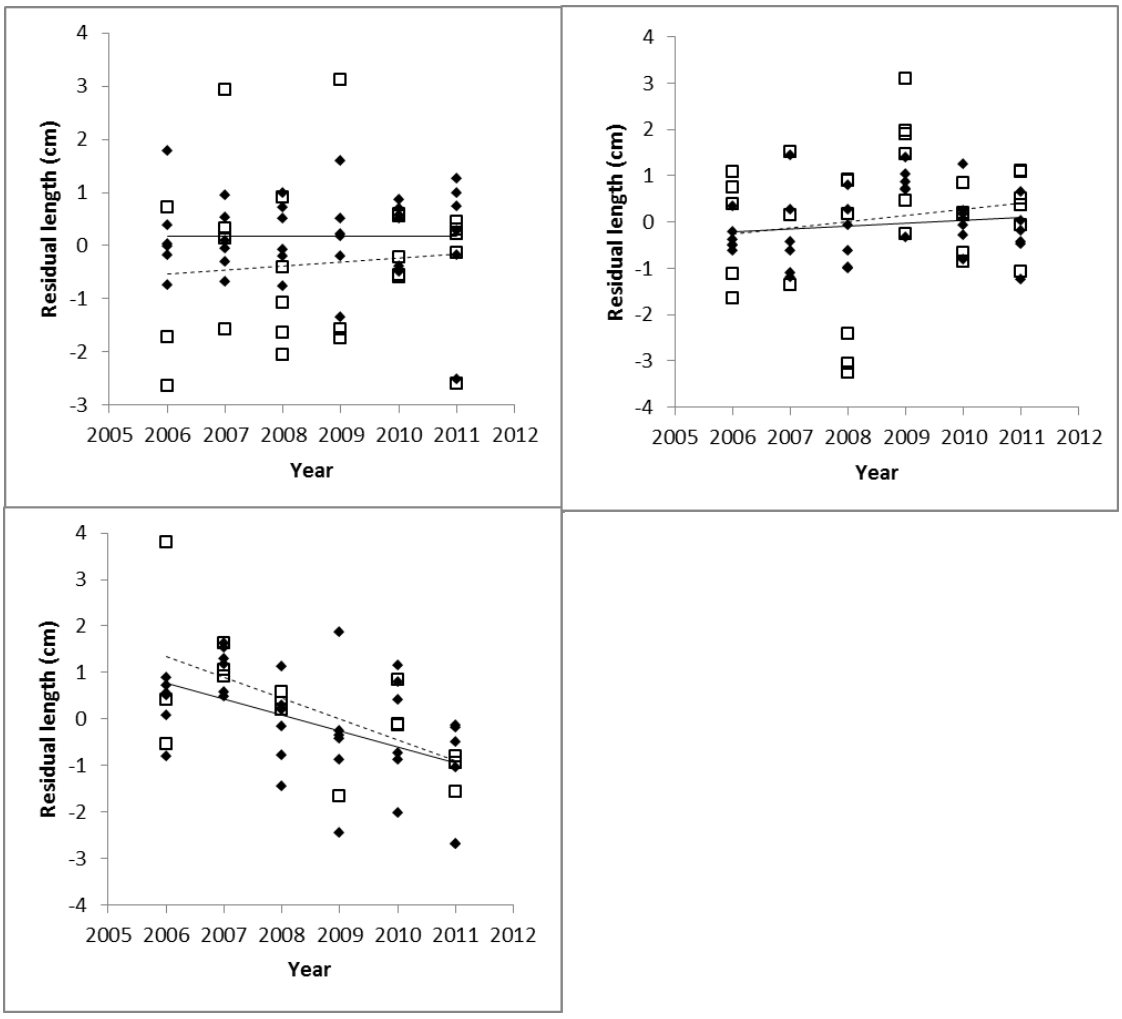
788 Figure 6.



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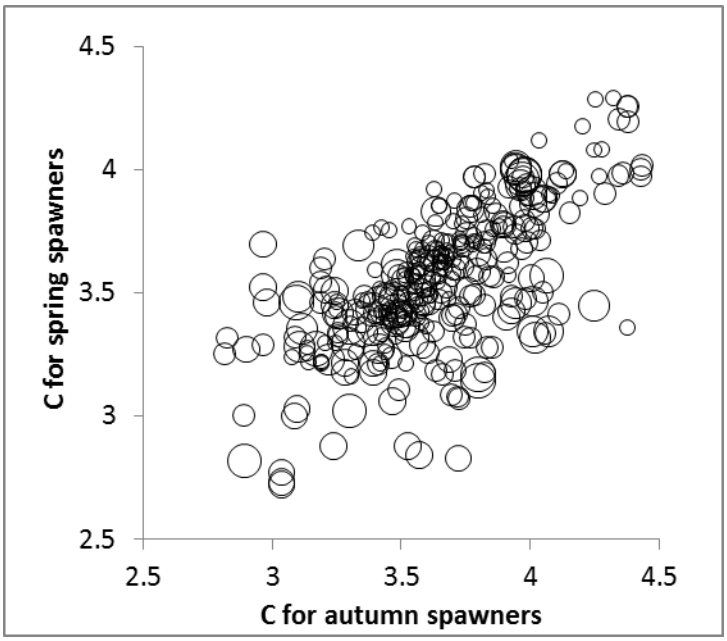
790 Figure 7.



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792 Figure 8.

Chapter 4: The structure of the community of herring in the Study area

What structures the herring soup 'stock'?

Paper 4

Divergent origins of sympatric herring population components determined using genetic mixture analysis

Dorte Bekkevold, Lotte A. W. Clausen, Stefano Mariani, Carl André, Tina B. Christensen, Henrik Mosegaard

The origin and reproductive interactions of sympatric, spatially separated spawning components of Atlantic herring *Clupea harengus* have received long-standing interest. In the western Baltic most herring spawn in spring, with smaller components spawning in winter. We used microsatellite DNA analysis and a novel Bayesian genetic mixture analysis approach to compare the genetic relationships of 2 western Baltic winter-spawning aggregations with those of their sympatric spring-spawning components, and combined information for genetic markers and morphological traits (otolith-determined hatching time and growth relationships) to test alternative hypotheses for the origin of winter spawners. We show that genetic relationships between sympatric components differ greatly between the 2 locations; the results indicate that winter spawning has arisen via 2 fundamentally different processes: (1) as a result of 'spawning-time switching' in a local springspawning component and (2) via 1 or more founder events from an extant winter-spawning population into an area otherwise dominated by spring spawners.

Paper 7

Herring migration in the Western Baltic, Kattegat and Skagerrak; discovering divergent migration strategies using high resolution genetic markers, otolith markers and growth analysis

Lotte Worsøe Clausen*, Dorte Bekkevold*, Henrik Mosegaard, Anders Nielsen, Karl-Johan Stæhr, Tomas Gröhsler, and J. Rasmus Nielsen

*) contributed equally

Herring caught in the Western Baltic, Kattegat and Skagerrak representing several different populations belonging to the management stocks North Sea Autumn Spawners (NSAS) or Western Baltic Spring Spawners (WBSS). Combining data on population affiliation applying genetic markers, otolith microstructure and growth parameters the population complexity of the WBSS is explored. The spatio-temporal occurrence of the populations over the annual cycle is analysed with particular focus on the dominating population in the WBSS, the Rügen herring, and the potential structuring factors of the identified migration patterns are explored. The population complexity in the WBSS is confirmed and divergent migration strategies of the populations within the stock are identified. Of the WBSS, the Rügen herring display the longest migration distances, extending from the Western Baltic into the eastern North Sea during summer. The migration strategy appears to be driven primarily by growth potential of the individual herring. The other populations managed as the WBSS stock do to some degree conform to the same migration strategy, but appear more locally bound and not migrating vast distances. The persistence of the observed genetic population differentiation is seemingly linked to divergent migration strategies and environmental heterogeneity e.g. in salinity. The management implications of the observed complexity in the spatial and temporal herring population occurrence in the area are discussed.

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Divergent origins of sympatric herring population components determined using genetic mixture analysis

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ABSTRACT: The origin and reproductive interactions of sympatric, spatially separated spawning components of Atlantic herring *Clupea harengus* have received long-standing interest. In the western Baltic most herring spawn in spring, with smaller components spawning in winter. We used microsatellite DNA analysis and a novel Bayesian genetic mixture analysis approach to compare the genetic relationships of 2 western Baltic winter-spawning aggregations with those of their sympatric spring-spawning components, and combined information for genetic markers and morphological traits (otolith-determined hatching time and growth relationships) to test alternative hypotheses for the origin of winter spawners. We show that genetic relationships between sympatric components differ greatly between the 2 locations; the results indicate that winter spawning has arisen via 2 fundamentally different processes: (1) as a result of 'spawning-time switching' in a local spring-spawning component and (2) via 1 or more founder events from an extant winter-spawning population into an area otherwise dominated by spring spawners.

KEY WORDS: Sympatric spawning · *Clupea harengus* · Microsatellite DNA · Spawning-time switching · Life history · Founder event · Assignment analysis

INTRODUCTION

Atlantic herring *Clupea harengus* are renowned both for their migratory and colonising capabilities and for large variations in morphology and life-history traits among stocks (Hay et al. 2001). Stock differences have been ascribed to a range of effects, spanning from phenotypic plasticity and transient subdivisions in an otherwise genetically panmictic species, to reproductively isolated, locally adapted population components (reviewed in McQuinn 1997a). Knowledge of demographic sub-structure has improved by replacing otolith macro- with otolith micro-structure analysis (McQuinn 1997b building on Messieh 1972 vs. Brophy & Danilowicz 2002 building on Mosegaard & Madsen 1996). Moreover, recent studies demonstrate low but significant genetic structure among Atlantic herring spawning components (McPherson et al. 2001, 2004, Bekkevold et al. 2005, Jørgensen et al. 2005, Mariani et al. 2005), rejecting panmixia and corroborating that, at least across large scales (e.g. seas, but also see Jørstad et al. 2004 for an example of structure on local scales),

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individual spawning components represent distinct populations. Genetic stratification is likely determined by mechanisms of natal homing, larval retention and possibly natural selection (e.g. Bekkevold et al. 2005).

Analyses of mixed-feeding and wintering schools show that spawning time often varies among stocks (e.g. in the Norwegian Sea: Husebø et al. 2005; the North Sea: Cushing 1967, Rosenberg & Palmén 1981, Hulme 1995; west of the British Isles: Brophy & Danilowicz 2002, 2003; and Gulf of St Lawrence: McQuinn 1997a). Individuals maturing in different seasons may even spawn at the same locations, and this phenomenon has been termed sympatric spawning with seasonal segregation (Winters et al. 1986). Two fundamentally different hypotheses can be invoked to explain the origin of sympatric components with divergent spawning times. The first, which has been coined 'year-class twinning' (McQuinn 1997b), entails a scenario by which juvenile growth coupled to variation in environmental conditions in some years causes fractions of individuals to mature and spawn in an earlier or later season than that in which they themselves were spawned (and hatched). Once individuals have switched they are expected to continue spawning in that season throughout their lives. In this case, seasonally separated sympatric components thus share population origin, and spawning time reflects a plastic response to external cues, operating under alternative reproductive strategies (cf. Gross & Repka 1998). In the second scenario, temporally divergent spawning components arise through founding events from populations exhibiting a different, be it genetically or environmentally determined, spawning season. In this scenario, sympatric components have different evolutionary origins and are expected to display genetic differentiation. The 2 hypotheses for establishment of spatially sympatric, temporally separated spawning components are thus testable, as distinguishing between them can be based on analyses of allele frequency differences.

Here, we determine genetic relationships and infer the most likely origin of 2 western Baltic winter-spawning components that occur sympatrically with larger spring-spawning components. We use microsatellite DNA analysis in conjunction with previously obtained data for major spawning components in the North Sea–Baltic Sea area and a novel Bayesian genetic mixture estimation approach by Pella & Masuda (2006). Their method provides a means for partitioning samples of individuals into baseline populations and putatively unsampled populations based on allele frequency information. Compared to other approaches the method has been shown to produce superior results under a range of scenarios (Pella & Masuda 2006), and the approach is ideal for our purpose as it allows us to test for the 2 aforementioned hypotheses (year-class twinning and immigration by founding event), as well as estimating the probability that the samples originated from 1 or more unsampled populations. Genetic results are compared with growth trajectories for individual spawning components, and results indicate that the examined spawning components likely arose via different processes.

MATERIALS AND METHODS

Sampling. Samples of herring *Clupea harengus* were collected in winter from 2 locations where temporally separated, sympatric spawning occurs. At Lillebælt, in inner-Danish waters, samples were collected in 2002 and 2003, and at Rügen, in the western Baltic, a sample was collected in 2004 (Fig. 1, Table 1). Among herring populations in the Northeast Atlantic, temporal separation in spawning time is observed among a large component in the English Channel spawning in winter (December and January), components in the western North Sea spawning in autumn (August to November) and components in the eastern North Sea, the Norwegian Sea, the Skagerrak, Kattegat, inner-Danish waters and the Baltic mainly spawning in spring (February to May) (ICES 1991). At both locations sampled in the present study, spawning thus otherwise mainly takes place in spring. While winter spawning is reported to occur regularly in both areas, the demography and temporal stability of winter-spawning components are not well described (Biester 1979). Although winter and spring spawners at Lille-

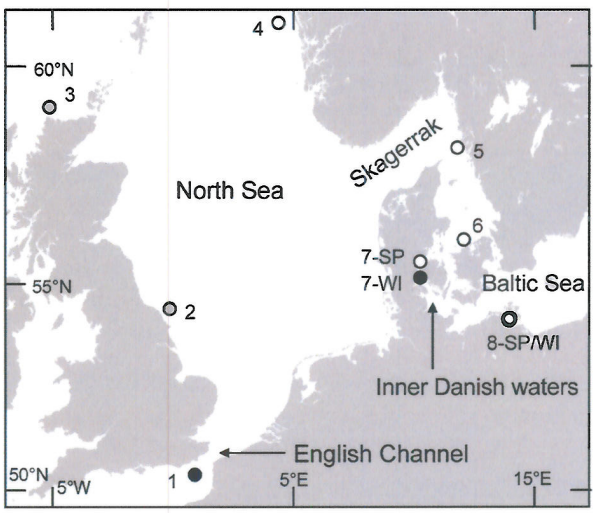

Fig. 1. *Clupea harengus* sampling locations (sample numbers refer to Table 1) (open, black and grey symbols: spring, winter and autumn spawners, respectively)

Table 1. *Clupea harengus*. Samples used in the analysis. Age (mean ± SE) and sample size (N) are given with spawning and otolith-inferred hatching seasons. Sample locations, refer to Fig. 1. Details about Samples 1 to 3 are given in Mariani et al. (2005) and about Samples 4 to 6, 7-SP and 8-SP in Bekkevold et al. (2005). SP: spring; WI: winter

Location	Sample no.	Spawning season	Hatching season	Sampling date	N	Age
English Channel	1	Winter	Winter	20 Nov 2003	99	3.91 ± 0.12
Flamborough	2	Autumn	Autumn	17 Sep 2003	96	3.39 ± 0.15
Cape Wrath	3	Autumn	Autumn	23 Aug 2003	112	3.38 ± 0.11
Møre	4	Spring	Spring	17 Feb 2003	78	7.74 ± 0.20
Skagerrak—Flatbrotten	5	Spring	Spring	19 Mar 2003	100	3.95 ± 0.11
Kattegat	6	Spring	Spring	3 Apr 2003	100	4.40 ± 0.11
Lillebælt	7-WI02	Winter[a]	Winter	11 Nov 2002	77	3.95 ± 0.14
	7-WI03	Winter[a]	Winter	23 Nov 2003	77	3.14 ± 0.06
	7-SP	Spring	Spring	7 Apr 2003	100	5.79 ± 0.15
Rügen	8-WI	Winter	Spring	7 Dec 2004	100	4.18 ± 0.11
	8-SP	Spring	Spring	24 Apr 2003	100	5.72 ± 0.14

[a]Inferred from maturity stage at time of sampling

bælt were collected from locations separated by 30 km, the location sampled in winter also acts as a spawning location for spring-spawning components, assumedly representing the same population as the analysed spring spawners. The maturity stage of each fish was recorded using a standard maturation scale (ICES 1962), and the hatching season (the season in which the fish was born) was determined from otoliths using a visual inspection procedure that has been shown to perform reliably across samples and readers (Clausen et al. 2007). The latter examinations revealed that the Lillebælt collections consisted of fish with different hatching times (winter, spring and autumn). However, in both years, the majority of sampled herring was winter spawned (70 and 68% in 2002 and 2003, respectively). Furthermore, as the spring- and autumn-spawned herring were less mature, they were expected to represent migrants rather than spawners and were therefore excluded from the analyses. Standard length was recorded for each fish, and the number of otolith winter rings was determined using the procedure described in ICES (2003) and entered as a proxy for age.

Molecular analysis. DNA was isolated from fin tissue using a Chelex technique (Walsh et al. 1991). A suite of 9 tetranucleotide microsatellites, corresponding with those analysed by Bekkevold et al. (2005) and Mariani et al. (2005), were PCR amplified, and fragment sizes were screened using a BaseStation 51 fragment analyser (MJ Research) in conjunction with the software Cartographer 1.2.6 (MJ Geneworks) under the conditions described in Bekkevold et al. (2005).

Baseline samples. Baselines used to determine the most likely population origin of samples were collected and analysed in connection with the genetic studies reported in Bekkevold et al. (2005) and Mariani et al. (2005). Between these 2 studies, herring were collected from a total of 18 spawning locations spanning the North Sea, Skagerrak, inner-Danish waters and the western Baltic Sea. Samples analysed in these studies represent the area's major populations, and were previously shown to exhibit genetic relationships that were temporally stable and to conform to an isolation-by-distance model. Genetic differentiation varies within and among seas, with spawning components in the North Sea and English Channel exhibiting close genetic relationships (F_{st} estimated at 0.001; Mariani et al. 2005) and components spanning the North Sea–Baltic Sea transition zone exhibiting higher differentiation (F_{st} estimated at 0.008; Bekkevold et al. 2005). Genetic information for populations of spring-spawning herring from Lillebælt and Rügen (Bekkevold et al. 2005) and for the winter-spawning component from the English Channel (Mariani et al. 2005) was compared to the western Baltic winter-spawning components sampled for the present study. Moreover, in order to examine the genetic resolution in the data, we used genotype information for populations at Møre, Cape Wrath and Flamborough (representing, respectively, eastern, western and central North Sea populations), in the Skagerrak and Kattegat and in inner-Danish waters (Table 1). These samples provided good representation of the area's population genetic structure (Bekkevold et al. 2005, Mariani et al. 2005) and cover populations potentially occurring in the western Baltic (see below). Genotype data were calibrated among all samples and were comparable over the full geographic scale (Ruzzante et al. 2006). To ensure consistency in scoring of microsatellite fragment sizes between studies, the same set of standard individuals was run on all gels.

Analysis of genetic variation. Overall heterozygosity and allelic richness were estimated for the 3 winter-spawning samples and compared with estimates for

the baseline samples previously reported in Bekkevold et al. (2005) and Mariani et al. (2005). Allelic richness was estimated using a rarefaction method, implemented in the software FSTAT (Goudet 2001). To illustrate genetic relationships among samples, F_{st} was estimated by θ for all sample pairs following Weir & Cockerham (1984), and statistical significances were evaluated by permutation tests using FSTAT run with 10 000 replicates. Genetic relationships were visualised applying multidimensional scaling (MDS) analysis, implemented in ViSta (Young 1996), of the matrix of pair-wise F_{st} estimates.

Genetic mixture analysis. To examine genetic relationships of the winter samples with those of the major spawning components in the area, we used a novel Bayesian Monte-Carlo Markov Chain (MCMC) method developed by Pella & Masuda (2006) and implemented in the software HWLER. The approach uses genotype information to partition samples of unknown origin into subsets of individuals from known (baseline) populations and from unknown (extra-baseline) populations by grouping individuals so that Hardy-Weinberg and linkage disequilibrium conditions are satisfied. The probability that individuals of unknown origin represent 1 or more genetically distinct populations not included in the baseline is gauged, along with the most likely population origin of each individual. Analyses were carried out for each of the 3 samples (Rügen 2004 and Lillebælt 2002 and 2003), following recommendations in the HWLER manual. To assess the statistical robustness of the HWLER approach, we carried out an additional analysis, in which Kattegat individuals (Sample 6 in Table 1) were entered as having unknown origin and tested against a baseline comprising the remaining 7 populations. This population was chosen as its genetic differentiation from Lillebælt spring spawners was estimated at roughly the same magnitude as that between English Channel and Lillebælt spring spawners (see below). Finally, we carried out 2 analyses in which either the Lillebælt 2002 or 2003 sample was used as an additional, separate population sample in the baseline, and the Lillebælt 2003 or 2002 sample was entered, respectively, as having unknown origin.

For each analysis, HWLER was run for 420 000 MCMC partitions thinned by 4; the second halves of chains were used to assess κ, the posterior number of populations represented among samples of unknown origin. The convergence of chains after burn-in was assessed by checking consistency in binary trees based on, respectively, the first and second halves of chains obtained after burn-in, using PartitionView (available at www.univ-montp2.fr/~genetix/partition/partition.htm) in conjunction with NJplot (available at http://pbil.univ-lyon1.fr/software/njplot.html). HWLER was also used to estimate the probability of each individual originating in each of the baseline populations or 1 or more of the potential unsampled populations.

Growth analysis. To assess growth patterns for the different components, length-at-age was compared between sympatric samples using ANCOVA of log-transformed total body length on log-transformed age estimates. In these analyses information for Lillebælt winter spawners was combined for the 2 sampling years, as the 2003 sample mainly represented a single year class.

RESULTS

Maturity and otolith analyses

All individuals in the Rügen sample were ripe-and-running spawners, whereas both Lillebælt samples consisted of mature (Stage 5) individuals that were not yet spawning, but could be assumed to do so within <1 to 2 mo (see 'Discussion'). All Lillebælt individuals in the analysis were winter hatched and thus showed correspondence between hatching and spawning seasons. In contrast, the Rügen sample consisted entirely of spring-hatched individuals, and thus represented individuals that spawned in a season different from that of their parents.

Sample genetic differentiation

High genotyping success was observed in the 3 winter-spawning samples, as scoring success across 9 loci in 254 individuals was 99.15%. Observed heterozygosity and allelic richness, along with pair-wise F_{st} values, are given for samples in Table 2, and an MDS plot illustrating genetic relationships is shown in Fig. 2. The MDS analysis indicated that the Rügen winter-spawning sample grouped with their sympatric spring spawners (and with Lillebælt spring spawners), whereas both Lillebælt winter-spawning samples grouped with the geographically distant English Channel population, and not with their sympatric spring-spawning Lillebælt component. The 2 Lillebælt samples exhibited low differentiation that was statistically significant prior to correction for multiple tests and were therefore analysed both separately and as a pooled sample in the genetic mixture analyses.

Genetic mixture analyses

In both analyses involving the Lillebælt winter-spawning samples, HWLER returned low probabilities for the samples originating from 1 or more unsampled

Table 2. *Clupea harengus*. Sample heterozygosity (H_o) and allelic richness (r) across 9 microsatellite loci, and pair-wise genetic differentiation estimated by F_{st} (above diagonal); p-values for tests for differentiation are given below diagonal. Significance following sequential Bonferroni correction (k = 55) is shown by asterisks. ***p < 0.001; **p < 0.01; *p < 0.05. Comparisons between sympatric components are underlined

Sample (sample no.)	H_o	r	(1)	(2)	(3)	(4)	(5)	(6)	(7-SP)	(7-WI02)	(7-WI03)	(8-SP)	(8-WI)
English Channel (1)	0.812	15.31		0.0018	0.0024	0.0021	0.0025	0.0147***	0.0157***	0.0025	0.0055***	0.0169***	0.0143***
Flamborough (2)	0.820	14.75	0.3520		0.0000	0.0022	0.0039	0.0179***	0.0177***	0.0051	0.0066*	0.0174***	0.0134***
Cape Wrath (3)	0.801	14.70	0.1160	0.5853		0.0014	0.0038**	0.0164***	0.0157***	0.0034	0.0050***	0.0161***	0.0127***
Møre (4)	0.842	14.28	0.3998	0.0514	0.1229		0.0048**	0.0193***	0.0182***	0.0051*	0.0077***	0.0183***	0.0141***
Skagerrak (5)	0.811	14.98	0.0097	0.0303	0.0002	<0.0001		0.0116***	0.0090***	0.0032*	0.0054***	0.0120***	0.0105***
Kattegat (6)	0.810	12.99	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.0021	0.0125***	0.0172***	0.0039***	0.0047***
Lillebælt spring (7-SP)	0.832	13.30	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0051		0.0125***	0.0172***	0.0020*	0.0017
Lillebælt winter 2002 (7-WI02)	0.787	15.50	0.0293	0.0192	0.0181	0.0006	0.0021	<0.0001	<0.0001		0.0047	0.0109***	0.0114***
Lillebælt winter 2003 (7-WI03)	0.805	14.51	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0088		0.0194***	0.0174***
Rügen spring (8-SP)	0.793	12.96	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0061	<0.0001	<0.0001	0.0003	
Rügen winter (8-WI)	0.817	13.16	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0337	<0.0001	<0.0001	0.1888	

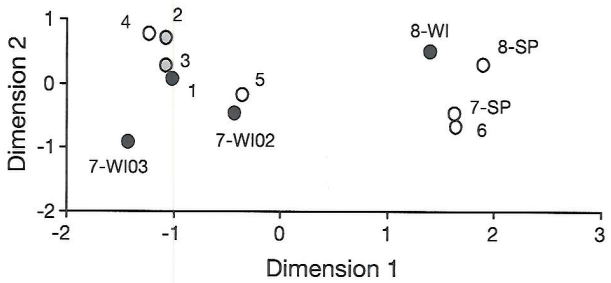

Fig. 2. *Clupea harengus*. Multidimensional scaling plot of the first 2 dimensions (describing, respectively, 70 and 9% of variation; stress = 0.071) for pair-wise F_{st} values for western Baltic winter-spawning and baseline samples. Sample numbers, refer to Table 1 (open, black and grey symbols: spring, winter and autumn spawners, respectively)

baseline populations (p[κ > 0] = 0.11 and 0.02, for 2002 and 2003 samples, respectively), indicating that all individuals likely originated from one of the baseline samples. The assignment analyses indicated that individuals sampled in 2002 were genetically similar to English Channel winter spawners, as 51 of 77 fish assigned to this population at an average probability of 0.56. Nineteen fish assigned to the North Sea population Cape Wrath (at average p = 0.55) and the remaining 7 were assigned among baseline samples Møre, Flatbrotten, Lillebælt and Rügen at fairly low probabilities (average p = 0.39). All individuals in the 2003 sample were assigned to the English Channel at p > 0.93. When Lillebælt 2002 and 2003 samples were pooled and entered as a single unknown sample, results were consistent, as p[κ > 0] = 0.06. Again, individuals most likely originated in the English Channel (at average p = 0.79, with only 1 of 155 multi-locus genotypes being slightly more likely in the Cape Wrath sample compared to English Channel [p = 0.51 and 0.44, respectively]).

HWLER did not return evidence that individuals in the Rügen sample originated from a distinct, unsampled population either, as p[κ > 0] = 0.06, but in contrast to Lillebælt, Rügen winter spawners assigned to their sympatric population (100 fish at average p = 0.66, with individuals alternatively assigned to the Lillebælt spring-spawning population at average p = 0.33). When Kattegat fish were entered as having unknown origin, the algorithm successfully recognised the presence of 1 or more unsampled populations, with highest probabilities for 2 or 3 unsampled populations (p[κ = 0] = 0.01, p[κ = 1] = 0.08, p[κ = 2] = 0.45, p[κ = 3] = 0.35, p[κ >3] = 0.11).

When Lillebælt winter spawners from 2002 were included as an additional baseline population and Lillebælt 2003 individuals represented the unknown sample, the algorithm produced ambiguous results.

The posterior probability was highest for a single unknown population (p[κ > 0] = 81%, with p[κ = 1] = 75%). However, in the individual assignment analyses, only 3 fish most likely originated in the suggested unsampled population (assigned at average p = 0.81), and the remaining fish most likely originated in the Lillebælt winter-spawning 2002 sample (58 fish at average probability 0.76), the English Channel population (10 fish at average p = 0.52), or in other populations (6 fish at average p = 0.41). In contrast, when the 2003 Lillebælt sample was included in the baseline and run against individuals sampled in 2002, results indicated low probability for 1 or more unsampled baselines (p[κ > 0] = 0.12, with p[κ = 1] = 0.11), and that 2002 individuals most likely originated in the English Channel, Cape Wrath, or Lillebælt 2003 (36, 32 and 31% at average probabilities of 0.57, 0.43 and 0.56, respectively), demonstrating low power for distinguishing among individual origins in these 3 baseline samples.

Table 3. *Clupea harengus*. ANCOVA results for differences in intercepts and slopes of (log-log transformed) length-at-age in samples

	Intercept			Slope		
	F	df	p	*F*	df	p
Lillebælt winter vs. spring	84 821.1	247	<0.001	0.753	246	0.387
Lillebælt winter vs. English Channel	50 324.9	245	<0.001	5.329	244	0.022
Rügen winter vs. spring	42 470.0	194	<0.001	0.201	193	0.655

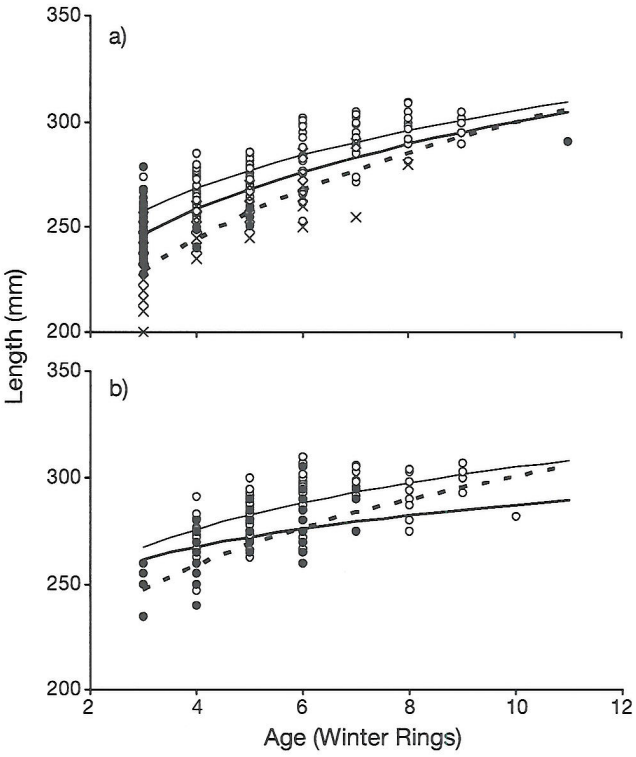

Fig. 3. *Clupea harengus*. Length-at-age relationships and estimated regression lines (for log-log transformed data). (a) Lillebælt winter 2002/2003 (●, bold line), Lillebælt spring (○, fine line) and English Channel (×, dashed line). (b) Rügen winter (●, bold line) and Rügen spring (○, fine line). The regression line for Lillebælt winter 2002/2003 is also shown for comparison (dashed line)

Population-specific growth patterns

All length-at-age comparisons showed highly significant differences in intercepts for the estimated relationships, but only Lillebælt winter spawners and English Channel winter spawners exhibited significant differences in estimated slopes (Table 3). In all other comparisons, slopes were not significantly different, indicating that growth patterns were similar for winter- and spring-spawning components (Fig. 3).

DISCUSSION

Using a Bayesian approach for assessing genetic relationships in samples of unknown population origin, we demonstrated that 2 western Baltic herring *Clupea harengus* winter-spawning components from an area of predominant spring spawning exhibited fundamentally different genetic relationships with their sympatric components. Rügen winter spawners, which themselves were spring hatched, closely resembled their sympatric spring-spawning component with respect to both allele frequencies and growth patterns. The combined genetic and morphological analyses thus indicated that the Rügen winter-spawning component had arisen through spawning-time switching in local spring-spawned herring, corroborating that spawning time is not genetically predetermined. Several otolith-based analyses have shown that individuals hatched in one season may spawn in another (e.g. Aneer 1985, McQuinn 1997b, Brophy et al. 2006), but also that spawning-time fidelity is prevalent for East Atlantic herring (Husebø et al. 2005, Brophy et al. 2006, Clausen et al. 2007). Although juvenile growth rate is known to influence age-at-maturity, the general consequences for spawning season are not clear (compare McQuinn 1997b with Brophy & Danilowicz 2003), and proximate determinants of spawning-season switching have not been identified. Contrary to expectations under a hypothesis of spawning time deter-

mined by growth conditions (e.g. McQuinn 1997b), growth trajectories were similar for spring and winter spawners, suggesting that individuals experienced similar environmental conditions and/or growth responses during (parts of) their life cycles. It has not yet been possible to determine whether winter spawning is recurrent in the area, or thus whether the sampled winter-spawning component is transient or ecologically stable over time.

In Lillebælt, analyses of temporally repeated samples from a winter-spawning component demonstrated lack of genetic correspondence between sympatric spring and winter spawners. Whereas Lillebælt spring spawners show a genetic relationship with neighbouring spring-spawning populations in the western Baltic (Bekkevold et al. 2005), winter spawners in contrast exhibited a close genetic relationship with the English Channel component. Winter-spawning components also occur west of the British Isles, but English Channel herring represent the only major winter-spawning component likely to be present in the western Baltic (ICES 1991). Following hatching, North Sea autumn-spawned and English Channel winter-spawned larvae drift into the Skagerrak, where they feed for 1 to 2 yr before migrating back to spawn in natal areas. Munk & Christensen (1990) showed that autumn-spawned larvae drift east and enter the Skagerrak–Kattegat area 4 to 5 mo post-hatching. The drift pattern for English Channel herring correspondingly describes a northeasterly direction (Bückmann & Hempel 1958), but whether larvae can drift as far as the western Baltic has to our knowledge not been investigated. We were not able to directly determine whether the Lillebælt winter spawners were born locally or elsewhere, drifting or actively migrating into the area. However, we found evidence that different year classes dominated in successive years (Table 1), indicating that their presence was a recurrent phenomenon. All Lillebælt winter spawners were Stage 5 and none was ripe-and-running (Stage 6) at the time of sampling. Though herring may retain maturity Stage 5 for several months depending on temperature, body size and condition (Bowers & Holliday 1961, Iles 1964, Lambert 1987), typical behaviour is to congregate at spawning locations days to weeks prior to spawning (Haegele & Schweigert 1985). Individuals were collected in a known winter-spawning area (Jensen 1949), approximately 1500 km (shortest water-way distance) from the English Channel, during that population's spawning season. Herring in Stage 5 in autumn (November) are, moreover, likely to display a short maturation cycle and spawn shortly after reaching full maturity (Bradford & Stephenson 1992). It thus seems reasonable to assume that individuals eventually spawn at the location, although timing only could be determined to within 1 or 2 mo (i.e. December and January). Moreover, the fact that their growth rates (Fig. 3) differed from those of English Channel spawners, but not from sympatric spring spawners, indicated that the Lillebælt winter spawners had experienced environmental conditions similar to those of sympatric spawning components and dissimilar from those of English Channel spawners.

The 2 Lillebælt samples exhibited marginally significant differentiation in allele frequencies between the 2 sampling years, and, although both samples grouped with the English Channel in the MDS analysis (Fig. 2), the 2003 sample exhibited statistically significant differentiation from this population (Table 2). Moreover, the HWLER analyses indicated that, whereas the 2002 sample was overall similar to the English Channel population, the 2003 sample tended to be more closely related to the Lillebælt 2002 sample. A possible explanation for this initially puzzling result is related to error associated with sampling a limited number of year classes (Jorde & Ryman 1995). Whereas the 2002 sample, which was not differentiated from its inferred population of origin, represented 3 main year classes, 92% of the individuals in the 2003 sample represented a single year class. Together with the very high resolution obtained with the chosen set of microsatellite markers (cf. Ryman et al. 2006), this unequal sampling of age classes may have led to the signal of significant allele frequency differentiation between temporal samples. Post hoc tests of pair-wise differentiation among year classes in the 2 samples returned no significant results (results not shown). The signal of differentiation between temporal samples is thus likely to be a combined result of sampling effects and of the high statistical power for detecting allele frequency differences, rather than evidence for different populations being sampled, or for the 2003 sample being affected by genetic drift. This was also suggested by the estimates of genetic variability, as all samples exhibited high levels of heterozygosity, and allelic richness corresponded between samples of presumed similar population origin (Table 2).

Herring spawning locations are widely distributed in the Northeast Atlantic (ICES 1991), and the applied baseline did not represent exhaustive sampling of components. However, the aim of the analysis was to test the hypothesis that sympatric, temporally separated spawning components are genetically related, rather than to examine the potential for assigning individuals to populations. Using mixed-stock simulations based on genotype information from the studies by Bekkevold et al. (2005) and Mariani et al. (2005), Bekkevold et al. (unpubl. data) examined the statistical power in mixed-stock analysis and effects of including or excluding multiple weakly differentiated baseline

population samples. They found that the probability for distinguishing among contributions from local (sub-) populations was low, and only on larger geographic scales (e.g. among seas) was statistical resolution adequate for partitioning stock contributions. They also showed that analyses incorporating baseline information for 9 vs. 15 populations generated consistent estimates of mixed-stock proportions, albeit confidence intervals narrowed when baseline sample numbers increased. Individual assignment procedures exhibit low statistical power under weak population differentiation (reviewed by Manel et al. 2005), and the approach is sub-optimal for determining mixed-stock proportions under most scenarios (Koljonen et al. 2006). In the present study, population differentiation was weak among North Sea and English Channel components, and between Rügen and Lillebælt spring spawners. This was directly reflected in the difficulty of assigning Lillebælt winter spawners to English Channel and North Sea baselines, and Rügen winter spawners to Rügen and Lillebælt spring-spawning baselines. Although individual assignment results should thus be interpreted with caution, their main merit was 2-fold. First, individuals collected together, in most cases, could be assigned to a common known baseline population and not an extra-baseline population, and, second, the most likely population of origin constituted that predicted from otolith data. The low power for distinguishing among origins in weakly differentiated populations meant that Rügen winter spawners potentially could represent immigrants from an unsampled weakly differentiated population and that Lillebælt winter spawners could have originated from North Sea autumn spawners that switched to winter spawning, although these present less parsimonious scenarios. Moreover, the analysis in which Kattegat fish were entered as an unknown sample unambiguously identified the presence of 1 or more genetically divergent components, although the algorithm, in line with results of other genetic approaches for estimating numbers of populations (reviewed by Waples & Gaggiotti 2006), exhibited low success in determining the actual number of extra-baseline populations. Nonetheless, these combined results showed that Rügen and Lillebælt winter spawners originated from populations that were genetically and presumably geographically close to, respectively, Rügen and English Channel herring.

The indication of a founder event from the English Channel (or western North Sea) to the Lillebælt raises questions about the frequency and ecological stability of immigrations. Range expansions and changes in the use of spawning locations have been reported for several herring stocks in the North Atlantic following fisheries-induced population collapses (reviewed in Corten 2001). Corten (2001) suggested that migratory changes are likely to occur when a year class recruits in the absence of older year classes, e.g. following fisheries depletion. In Corten's scenario, migratory routes are socially transmitted from older to younger fish. In the absence of the former, traditional routes are not transmitted to naïve recruits, which, as a result, may end up in novel spawning locations. Although the Lillebælt 2003 winter spawners were mainly from a single year class, the pooled Lillebælt samples collected over 2 consecutive years comprised several year classes, and it is therefore unlikely that they represented naïve recruits in line with Corten's scenario. The winter spawners may, however, be descendents of strayers that for some reason, be it related to effects of social learning, divergent growth, hydrographic features, or other factors, failed to home with other English Channel herring.

An important caveat when predicting effects of migratory behaviour on population structure is that observed frequencies of straying between populations need not reflect levels of reproductive isolation and gene flow. Hence, immigration success ultimately depends on selection pressures in the novel environment, as divergent local selection pressures may impede or prevent reproductive success in strayers (e.g. Rundle 2000). Immigrants and their descendents may reproduce successfully in some years, but show lack of persistence over ecological or evolutionary time scales. The spatially explicit genetic structure of herring populations in the North Sea–Baltic Sea area (Bekkevold et al. 2005, Jørgensen et al. 2005, Ruzzante et al. 2006) provides direct evidence that a significant degree of reproductive isolation is maintained over ecological and evolutionary time scales, although levels of gene flow vary across geographic scales. Within both the North and Baltic Seas population differentiation is, for instance, of comparatively lower magnitude than between the 2 seas and among populations in the transition zone. The 2 seas differ greatly in environmental conditions. The North Sea is a temperature-stable, saline (ca. 34) environment, whereas the Baltic Sea and the transition zone have more variable temperatures and are brackish, with salinities decreasing from 34 in the Skagerrak to almost zero in the Northeast Baltic. Proximate mechanisms restricting gene flow among herring populations have not been resolved, but patterns of genetic differentiation covary with salinity and temperature parameters (Bekkevold et al. 2005, Jørgensen et al. 2005), suggesting that local adaptation to these (or associated) environmental variables may be a factor. The observation that several fishes and other marine organisms in the North Sea–Baltic Sea area show population structuring at similar geographic levels (reviewed in Johan-

nesson & André 2006) further suggests a role for adaptive diversification in response to local salinity and/or temperature conditions across species. In connection with this, it is interesting that Lillebælt winter spawners seemingly spawn at much lower salinities (ca. 16) than English Channel herring (ca. 35)—their presumed population origin. Low salinity is known to reduce reproductive success in Pacific herring *Clupea pallasi* (Griffin et al. 1998), but it is yet unknown to which extent, e.g., English Channel herring would be reproductively impaired in a brackish spawning environment.

In conclusion, our analyses demonstrated that sympatric spawning herring components can exhibit divergent genetic origins and that combining genetic and morphological trait information presents a valuable means of determining the most likely origins of individual spawning components. Overall, our results yield a complex picture of previously not fully recognised biological diversity. In conjunction with recent demonstrations of spatially explicit stable population differentiation in Northeast Atlantic herring, it is however indicated that, although the observed life-history variation and plastic migratory behaviour lead to dynamic demographics and a high potential for gene flow, herring spawning components uphold significant levels of reproductive isolation, possibly affected by selective differences among spawning and/or larval habitats.

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Herring migration in the Western Baltic, Kattegat and Skagerrak; discovering divergent migration strategies using high resolution genetic markers, otolith markers and growth analysis

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Abstract

Herring caught in the Western Baltic, Kattegat and Skagerrak representing several different populations belonging to the management stocks North Sea Autumn Spawners (NSAS) or Western Baltic Spring Spawners (WBSS). Combining data on population affiliation applying genetic markers, otolith microstructure and growth parameters the population complexity of the WBSS is explored. The spatio-temporal occurrence of the populations over the annual cycle is analysed with particular focus on the dominating population in the WBSS, the Rügen herring, and the potential structuring factors of the identified migration patterns are explored. The population complexity in the WBSS is confirmed and divergent migration strategies of the populations within the stock are identified. Of the WBSS, the Rügen herring display the longest migration distances, extending from the Western Baltic into the eastern North Sea during summer. The migration strategy appears to be driven primarily by growth potential of the individual herring. The other populations managed as the WBSS stock do to some degree conform to the same migration strategy, but appear more locally bound and not migrating vast distances. The persistence of the observed genetic population differentiation is seemingly linked to divergent migration strategies and environmental heterogeneity e.g. in salinity. The management implications of the observed complexity in the spatial and temporal herring population occurrence in the area are discussed.

Keywords: Population complexity, migration, growth, structure

Introduction

Migration is frequently described as an adaptive, long-distance movement occurring predictably during a defined time-cycle, such as annually or during a full life-cycle. These movements take advantage of spatial and temporal differences in the distribution of resources (being food, reproductive habitat availability, shelter for predators, etc.), and thus increase the fitness of the migrants (Harden Jones 1976; Smith 1985; Chapman et al., 2012). When analysing a wide range of north temperate marine fish populations, Roff (1988) concluded that migratory species were relatively fast growing to a larger size, matured late and at a relatively large size, thus maximising their fecundity. However intraspecific trade-offs between growth, relative fecundity and migration are also common, e.g. in Atlantic herring (Silva et al., 2013). Divergent migration tactics are observed within a diverse array of fish species (Secor 1999), predominantly in many anadromous fishes (Jonsson and Jonsson, 1993). However, also many commercially important marine species have partially migratory populations (e.g. cod *Gadus morhua* (Cote et al., 2004). The spatio-temporal patterns of migrations within fish populations may vary over time for reasons not always clearly understood, such as changes in abundance, overexploitation of sub populations, or changes in environmental conditions (Fréon and Misund, 1999; Dingle & Drake, 2007; Melvin et al., 2009). Divergent migration patterns in terms of migration distance and timing may occur driven by e.g. fish age (Harden Jones, 1968; Dingle, 1996; Fréon & Misund, 1999), condition (Slotte, 1998b) or other factors (Jonsson & Jonsson, 1993; Chapman et al., 2012).

Atlantic herring is an abundant and widely distributed marine pelagic fish that spawns on substrate in coastal areas throughout most of the north Atlantic (Iles & Sinclair 1982). Most herring populations are migratory and often congregate on common feeding and wintering grounds where aggregations may consist of mixtures of individuals from several populations.

66 Large variation in morphology and life-history traits can be observed among herring stocks
67 (Hay et al. 2001). Herring are renowned for their plasticity, which has challenged population
68 identification and population delimitation has therefore been intensively studied (Geffen
69 2009). Differences among populations have been ascribed to a range of effects, spanning from
70 phenotypic plasticity and transient subdivisions in an otherwise genetically panmictic species,
71 to reproductively isolated, locally adapted population components (reviewed in McQuinn
72 1997). Knowledge of demographic sub-structure has improved through development of high
73 resolution stock discrimination methods applying otolith analysis (Mosegaard and Madsen
74 1996; Brophy and Danilowicz 2002, Clausen et al., 2007) and genetic marker analysis (e.g.
75 McPherson et al. 2001; Bekkevold et al. 2005; Jørgensen et al. 2005; Bekkevold et al., 2011).
76 Recently, genomic markers (Single Nucleotide Polymorphisms, SNPs) have been shown to
77 provide improved accuracy in genetic stock identification (Limborg et al. 2012; Nielsen et al.
78 2012). Genetic markers have also been applied to determine migration patterns in East
79 Atlantic herring (Bekkevold et al. 2007; 2011; Ruzzante et al. 2006). However, such techniques
80 have until now not been applied to assess migrations in the western Baltic Sea.
81
82 In the Western Baltic, Kattegat and Skagerrak a number of genetically divergent herring
83 populations with diverse spawning times and areas have been identified (Bekkevold et al.,
84 2005; Ruzzante et al., 2006). Thus a high degree of biocomplexity exits in the area (Ruzzante et
85 al., 2006), which only to a certain degree is considered by the management, given that all
86 spring spawning populations of herring make up the large 'management stock' defined as the
87 'Western Baltic Spring Spawners' (WBSS). Current fisheries management advice for TAC for the
88 entire Western Baltic-Kattegat-Skagerrak fishery is set at a level compatible with a
89 precautionary exploitation of the WBSS stock and is based on the assumption that one of the
90 spring spawning populations, the Rügen herring (RHS), constitutes the majority of spring-

91 spawning herring in the area (ICES 2010). Although studies tend to support the notion that
92 Rügen herring constitute an important component in the Skagerrak, they also show that the
93 proportion of spring- spawning herring in the Skagerrak represents an inaccurate proxy for the
94 size of the Rügen component (Bekkevold et al., 2011).
95
96 The preservation of intraspecific population integrity is a prerequisite for maintaining
97 population and life history diversity which in turn affect the performance of individual species
98 in providing important ecosystem services (Schindler et al., 2010). The resilience of a
99 population complex (or stock) is weakened if the population diversity within the population
100 complex is decreased and the dynamics of the populations become more synchronous (Hilborn
101 et al. 2003; Schindler et al., 2010). Understanding the spatio-temporal scale of population
102 structure and habitat use is a requirement for accurate assessment and sustainable
103 management of stocks. One possibility is to use spatial management measures (Kell et al.,
104 2009), but in order for this to be successful, knowledge of the migratory behaviour of the
105 populations within the stock and hence the degree of mixing in different areas is essential.
106 Given the current severely reduced stock size for WBSS (ICES 2013), knowledge of the
107 temporal and spatial distribution of its stock components is highly warranted. Such knowledge
108 is a prerequisite for adopting balanced management measures (Andersen et al., 2008) and can
109 pave the way for assessment techniques robust to temporal and spatial variation in the herring
110 population subjected to management.
111
112 This study applies genetic and otolith markers as well as morphological data to disentangle
113 migration patterns of the herring populations in the western Baltic. We analyse the migration
114 strategy of the RHS and discuss this in relation to occurrence of other genetically different
115 herring populations in samples taken over several seasons and locations within the Western

Baltic, the Kattegat and the Skagerrak. We examine if any life-stage or individual traits have an effect on the amplitude of the summer feeding migration and seek to clarify the hitherto only qualitatively described seasonal and age related pattern of the RHS migration. The RHS spawns in the Greifswalder Bodden off the German Rügen Island, predominantly during March-May (Klinkhardt, 1996; Figure 1). RHS is genetically distinguished from other herring in the North Sea-Baltic Sea area, and genetic marker data have in a single case been used to determine the tempo-spatial distributions of RHS in its main feeding area, the Skagerrak Sea (Bekkevold et al. 2011). RHS migration patterns within the Western Baltic and Kattegat, Skagerrak areas are qualitatively described in the literature (Aro 1989; Biester 1979; Nielsen et al. 2001; Otterlind, 1987) but has hitherto not been documented using genetic markers, nor contrasted with the other herring populations in the area. We examine whether any life-stage or other individual traits have an effect on the migration strategy in relation to spawning, feeding migration distance and clarify the hitherto only qualitatively described seasonal and age related pattern of the Rügen herring migration. The implications for a herring fishery management of the stock composition in terms of herring populations and their possible divergent migration patterns and thus mixing are discussed.

Methods

Sampling and background data

Based on a review of available data and information, sampling was done with the aim of covering major parts of the spatial and temporal distribution and stock composition. The nature of the RHS outmigration from spawning areas to the summer-feeding grounds in the Skagerrak and North Sea has earlier only been sporadically studied by mark-recapture experiments as the migration does apparently not happen in large condensed schools

(Klinkhardt, 1996), and no directed fishery is undertaken on these low-body condition post-spawners. To cover this part of the migration, recreational fishermen conducted a directed fishery on the out-migrating herring through all sounds in the Western Baltic (Figure 1). Additionally, data from monthly samples taken by the Thünen Institut für Ostseefischerei (OF) in Rostock of herring occurring in the Greifswalder Bodden in the period 1998-2005 were included to monitor the onset of spawning and the potential existence of spawning waves (Jørgensen et al. 2005; Stephenson et al., 2009). Herring were sampled monthly from February through May, recording age, length, weight, and maturity stage.

The data comprised both 1) baseline samples, which are samples of individuals collected during spawning time to represent all major reproductive units in a system, and 2) mixed-stock samples collected throughout the migration cycle. Mixed-stock samples were collected spanning from the onset of the post-spawning feeding migration through the Inner Danish waters to the Skagerrak, over the summer feeding and autumn back-migration, to the overwintering in the Sound and the Western Baltic (Table 1, Figure 1).

Spawning time baseline data

The classification of herring according to spawning time was based on baseline data from previous studies (Anonymous 2005) where otolith microstructure analysis (following the procedure in Clausen et al., 2007) was used to assess the season a herring was spawned, and presumably would spawn itself, i.e. 'spawning type'. Briefly, otoliths were first digitized using a Leica™ 350 F digital camera connected to a dissection microscope (Leica MZ6) and stored in an image database. Otoliths were then subjected to grinding and polishing, followed by a visual inspection to classify them as spring, autumn, or winter-spawner. In total, 571otoliths were examined from the spawning samples, and showed overall consistency in WBSS exhibiting spring-spawning and NSAS exhibiting autumn- and winter-spawning type classification (Table 2).

Genetic baseline data

Baseline genotype data for genetic stock identification, GSI, were obtained from previous studies. These studies explored population structure in Northeast Atlantic herring populations using SNP markers (Limborg et al. 2012), and SNP based assignment power (Bekkevold et al. *unpublished*) showed that populations in the focal area grouped into four main genetic clusters, to which individual assignment was generally accurate with 156 SNP markers. The four clusters corresponded to, respectively, 1) the NE Atlantic (including Norwegian spring-spawning and Icelandic herring), 2) W North Sea and populations spawning around the British Isles (i.e. including the NSAS), 3) the North Sea-Baltic Sea transition zone (mainly spring-spawning, including RHS) and 4) the Baltic Sea (mainly spring-spawning). In total, baseline data consisted of 773 individual genotypes from collections of 25-40 individuals representing a total of 23 spawning locations (Figure 1, Table 1), typed for 156 SNP markers (see Supplementary file for a list of genotyped markers). Levels of genetic variation were generally > 4 times larger among population clusters compared to among samples within clusters (Limborg et al. 2012). Within the North Sea/Baltic Sea transition area (from here on referred to as "W Baltic" for brevity), 9 of 28 population comparisons exhibited statistically significant differentiation in exact G tests (Bekkevold et al, *unpublished*), suggesting scope for assignment to sub-population within this area.

Supplementary data on spring spawning herring

The area where the Rügen herring are encountered (as part of the WBSS stock complex) is intensively monitored; five separate research-vessel surveys on herring in the area are undertaken yearly (Payne et al., 2009), the commercial catches are sampled intensively and thus the historical data available for the analysis of the migration pattern of RHS were ample. Further, a number of scientific cruises sampling herring cover different parts of the transition area during different parts of the year (Payne et al. 2009). Based on these historical records of

herring data on length, age, and spawning type from the data base on commercial and scientific biological samples from 1990-2012, a heat map of variation in growth potential among spring spawning herring in the distribution area was constructed. These 'supplementary data' thus contain no information about specific population origin, and should be considered as potential mixed-stock samples comprising all spring-spawning population components occurring in the respective areas. The maps were based on available information on size at age, expressed as residual size in relation to the average von Bertalanffy growth function for all genetically assigned WB-Rügen spring spawners (k=0.508, L_∞=28.80 cm, t_0=0). Data from scientific cruises were combined with otolith microstructure assigned spawner type to classify ICES rectangles during wintering (October-January), spring spawning (February-May) and summer feeding (June-September) as habitats for WB-Rügen spring spawners with different growth potential.

Statistical power for genetic stock identification

As we were here primarily interested in examining spatial distributions of RHS we initially explored the accuracy associated with GSI to population within the W Baltic cluster. We first performed a clustering analysis with the program *structure* (Pritchard et al 2000) using eight W Baltic samples and one sample from each nearest neighbouring cluster west (Central North Sea) and east (Gdansk) of the W Baltic (see Figure 1). The most likely number of clusters (K) in the data was examined repeating analyses for K = 1-6, allowing the model to include information on sampling location as a prior (Hubisz et al. 2009). Admixture proportions estimated per individual, as well as per sample were inspected for the suggested most likely value of K. Population admixture proportions were evaluated in combination with information about tests for pairwise sample differentiation. As results from these analyses indicated statistical resolution for distinguishing between RHS and other W Baltic populations (see Results) we designed a baseline for individual assignment of mixed-stock samples as follows:

the 20 population samples (Table 1) were grouped into five population sub-clusters, corresponding to respectively 1) the NE Atlantic, 2) the North Sea, 3) the Skagerrak/Kattegat/Inner Danish Waters (hereafter 'Transition'), 4) SW Baltic (Rügen & SW German fjords, hereafter 'RHS'), and 5) the Baltic Sea. Self-assignment analyses were then performed to these five sub-clusters using a 'leave-one-out' approach implemented in the package ONCOR (Kalinowski 2002). The test evaluates how well fish can be assigned to their population of origin and is conducted by removing fish one at a time from their baseline populations and then estimating their origin. Proportions of herring that assigned to another population cluster than that of sampling were used as estimates of GSI accuracy.

GSI of mixed-stock samples

RHS were identified from mixed-stock samples based on GSI for 21-86 fish per sample, depending on availability (Table 2). For all mixed-stock samples gill or fin tissue was upon collection stored in 96% ethanol. Genetic analyses of fisheries samples were performed using DNA extraction followed by PCR amplification and genotyping of 156 SNP markers, for which allele frequency information was already available for baseline samples. Genotyping was performed in 96.96 Dynamic Arrays using the Fluidigm IFC thermal cycler and BioMark instruments with SNPtypeTM chemistry. Genotypes were scored using the BioMark Genotyping Analysis software (Fluidigm, San Francisco, California, USA).

Mixed-stock samples were assigned origin using ONCOR and grouping baseline samples into five population sub-clusters, as described above. The applied assignment procedure uses estimates of stock mixture proportions to guide individual assignments, following Rannala and Mountain (1997). However, assignment was fully robust across methods, including the approach by Paetkau et al. (1997) (data not shown). Nonetheless, to minimise error associated with assigning individuals with genotypes of similar probabilities in more than one population cluster, only individuals coming out with a probability above 0.80 to one population cluster

were scored as assigned, excluding on average 20 per cent of individuals per sample from further analysis (see results).

Maturation stage, OM spawning type and growth of RHS from mixed stocks

For all sampled herring, growth was estimated using length at age, and maturity stage was recorded by visual inspection of gonads, as immature 1-2, maturing 3-5, spawning 6, or spent/recovering 7-8, using a standard maturation scale (ICES 1962). For graphical tabulation of maturities at distance to WB spawning grounds and month, stages 7-8 was assigned a zero and one.

Otolith microstructure analysis (OM) for spawner type assignment was carried out by visual inspection according to Clausen et al (2007). Comparison between assumed spawning period of genetically assigned mixed stock herring and OM assigned spawning type was analysed in contingency tables. A total of 571 genetically assigned herring were analysed for spawning type by OM visual inspection.

RHS growth approximation analysis

Standard length (cm) and the number of winter rings as a proxy for age (ICES 2012) was used in combination to analyse the life-stage of the individuals selected based on the above stock identification. To assess growth patterns for the life-stages in relation to their temporal-spatial distribution pattern, length-at-age was modelled using total body length on age estimates for each combination of time and location. Based on the representation of RHS in the samples, selected combinations of month:location was then analysed further for comparison of growth curves over the migration season; specifically the spawning event and the feeding migration were analysed for RHS and the latter compared with other spring spawning herring in the study area.

Identification of spawning waves

Morphological data from herring collected at the mouth of Greifswalder Bodden during spring (from February through May) from 1998-2005 was analysed comparing von Bertalanffy growth curves by month across all years. For each of the four spawning months (February through May across all sampling years) a von Bertalanffy growth curve with separate rate (K) and level (L_∞) parameters was estimated. The individual observations of length (L) for each age (A) were calculated as an average, but based on a different number of samples, N_i. This was accounted for by using a weighted nonlinear regression:

$$L_i = L_{\infty,m_i}\left(1 - e^{-K_{m_i}A_i}\right) + \varepsilon_i$$

where $\varepsilon_i \in N(0,\sigma^2/N_i)$ and i indicates observation number and m_i denotes month. Likelihood ratio tests were used to verify model reductions.

Comparisons of length-at-age by month and migration distance for spring spawning herring were done following a similar approach. For each observed combination of the five months six genetic groups a von Bertalanffy growth curve was estimated with separate rate (K) and level (L_∞) parameters. Since these are individual observations standard nonlinear regression was used:

$$L_i = L_{\infty,m_i,g_i}\left(1 - e^{-K_{m_i g_i}A_i}\right) + \varepsilon_i$$

where $\varepsilon_i \in N(0,\sigma^2)$ and g denotes the Genetic assigned population and the remaining variables are as above (Table 2).

Analysis of migration

Given the sampling design for the genetic analysis, the autumn and winter seasons had relatively few available samples, thus data on spring spawning herring (assigned to spawner type based on otolith microstructure) from all available cruises in the study area was applied

to analyse the yearly cycle of migration of the RHS. The growth potential in herring spring spawners caught in scientific cruises was derived as residuals to a general von Bertalanffy growth curve for genetic defined RHS (k=0.508, L_∞=28.8025, t_0=0) and plotted by ICES rectangle for three seasons; winter (October to January), spring (spawning time, February to May), and summer (feeding; June to September). Using the 'supplementary data' samples taken with different spatial resolution could all be assigned to ICES statistical rectangle and an index of distance D, between spawning grounds in the Western Baltic and the summer feeding grounds to the north in the Kattegat, the Skagerrak and the eastern North Sea was calculated on an ICES rectangle basis based on the schematic diagram shown in Figure 2.

The distance measure, D, was used to analyse the spatial and temporal signals of maturity and size at age of the migrating spring spawning herring. In total, 54 squares were analysed from the period 1998-2012, and analyses comprised information for an average of 206 fish per ICES rectangle, per season (std=328).

Results

The results are presented in two parts; first identifying the herring populations present in the study area both at spawning time and in mixed aggregations during migratory stages. Secondly, we present the results related to the migration strategy of the RHS. Thirdly, we present growth results building on the 'supplementary data' comprising combined information for all spring-spawners recorded in the study area.

Population identity analyses; herring populations in the Study area

A total of 571 herring were subjected to otolith analysis, genotyping and morphological analysis from samples covering the study area (Figure 1).

Genotype results

Structure analyses returned K=3 to be most likely model, indicating that the W Baltic baseline data were best described using a model with three genetic clusters (Supplementary Figure). Individuals and samples exhibited admixed clustering patterns, indicating somewhat low resolution for genetic classification. Nonetheless, SW Baltic samples (including RHS and the two neighbouring populations Schlei and Kiel) clearly exhibited different clustering patterns from all more northern populations from the Transition area, suggesting power to differentiate between RHS and neighbouring fjords (hereafter collectively referred to as RHS) and other Transition populations. In concordance, genetic self-assignment tests showed that assignment accuracy varied among population clusters, with NE Atlantic, North Sea and Baltic Sea herring generally assigning correctly, whereas RHS and Transition herring in respectively 13 and 17% of the times mis-assigned to each other (Table 3), reflecting the somewhat low levels of differentiation among these neighbouring populations. An error rate of 17% was therefore applied to estimates, where the relative proportions of Transition versus RHS herring were considered.

Mixed-stock samples genotyping success was overall high at 97% loci scored across loci and individuals. In total, 78% of 859 genotyped individuals were assigned origin (Table 2). In one sample of 69 fish from the N Kattegat, 46% could not be assigned. Half of these fish (18 of 32) exhibited genotypes with almost similar probabilities in RHS and Transition, reiterating that statistical power was sometimes low for distinguishing between these sub-populations. There were considerable differences between stock compositions across the examined areas from the Skagerrak to the W Baltic. Proportions of RHS herring varied between 15-88% among the 16 collections, and in 69% of samples RHS herring dominated among contributions from the W Baltic and the Baltic Sea, corroborating the importance of this sub stock to fisheries. As

expected, North Sea herring were mainly encountered in the Skagerrak samples and Baltic Sea herring mainly in the W Baltic samples.

Otolith and Morphological analysis

The results of the analysis of otolith assigned spawning type, maturity stage, and growth analysis are described under the sections where these results are used as supporting information underlying the genetic results outlined above. Given that the application of otolith assigned spawning type was used both as supplementary analysis to the genetic marker analysis but also as stand-alone markers of spawning type, a correlation between the two assignment techniques was done. In the text-table below, the otolith assigned spawning type is matched with the genetically assigned population for the same individual. Table 4 shows the cross-tabulated comparison of genetic assignment and assumed spawning time with otolith microstructure assignment to spawning time.

RHS, Baltic, and Norwegian Spring Spawners all exhibit good correspondence with assumed spawning time for the respective herring populations. Skagerrak also assigns with the expected proportions, whereas North Sea herring assigns most individuals to the autumn spawner type and somewhat less to winter spawners however it also has a proportion of spring spawners as identified by the otolith microstructure. The deviation from the genetic assigned population probably originate in somewhat overlapping spawning seasons (e.g. late spawned autumn) and/or methodological errors (e.g. overgrinding of otoliths, Clausen et al., 2007).

Migration strategy analyses

These analyses were done specifically for RHS following the population from spawning around the Rügen Island through the summer feeding-migration to the autumn/winter period. However, to disentangle potential diverging migration strategies between the herring

populations in the area, the most likely migration patterns of the other herring populations in the area were also examined.

Spawning

Maturity stages of spring-spawning herring (based on otolith marker analysis) older than 2 wr were collated from the supplementary data. Peak observed maturity (mode) was calculated and tabulated (Table 5), in a matrix of month and distance D. A spatio-temporal signal of maturing individuals can be followed from the North to the South starting at the longest distance from the Western Baltic spawning grounds in September, with maturity increasing in all areas (D=2-15) from October to February and concentrating in the southern areas in March, until maturity peaks close to the spawning grounds in March-April with actual observations of ripe and running herring. In April-May both maturing and spent herring are found, whereas June-August clearly is a recovery period in all areas.

The analysis of spawning individuals during the spawning months (February through May) clearly demonstrated the existence of spawning waves. Likelihood ratio test for common rate parameters (same k for all months) was accepted with a p-value of 98% and likelihood ratio test for common level was rejected with a p-value <0.0001 (Table 6). The growth curves for the four different months were significantly different w.r.t. the L_∞ (Figure 3, legend; Table 6). The final estimated curves are seen in Figure 3. The difference in growth trajectories between the herring spawning early and late in the season was most pronounced in the older individuals.

Feeding migration

Following the RHS over an entire yearly cycle of migration was not possible given the missing months in the sampling design, however, when analysing the growth potential by month and area of the genetically assigned spring spawning populations at a higher resolution (but with

379 very low power; RHS: RUG, SCHLEI, KEIL and Transition: SKA, KAT, IDW), a migration strategy
380 was indicated (Figure 4). In February, samples taken close to the spawning site showed RHS
381 (here annotated with RUG) with a higher length at age than individuals belonging to other
382 spring spawning populations, (Figure 4 top left panel), however, when sampled in the middle
383 of the spawning season, the larger RHS were not found in the samples (Figure 4, top middle
384 panel; April).
385 The samples taken in the early summer (June) in the main feeding area in the Skagerrak (Figure
386 1), contained RHS with a larger length at age compared to the Transition populations (Figure 3,
387 lower left panel), where the samples taken later in the summer (July) showed no such
388 difference, indicating that the faster growing individuals had moved out of the area (Figure 4,
389 lower right panel).
390 The apparent differences in growth, as evaluated by the length-at-age by genetically assigned
391 population (Figure 4), indicated that the RHS (RUG in Figure 4) migration pattern was best
392 described by the growth potential. Figure 5 summarise the migration strategy of WBSS and
393 other spring spawning components.
394 The migration distance increases with growth potential as interpreted by the analysis of von
395 Bertalanffy outliers. This growth potential determined spatial distribution was most
396 pronounced during spring and summer, where the spring spawners with the highest growth
397 potential were found in the outermost Skagerrak and the Eastern North Sea. Extrapolating
398 from the population assignment results (Table 2) it is expected that RHS make up more than
399 half of the spring-spawning herring in these areas, and it is therefore expected that the
400 elevated growth rates is associated with presence of fast-growing RHS.
401 The spring-spawning herring caught in the Western Baltic exhibited negative residuals in all
402 seasons, indicating that they apparently had lower growth potential than immigrating
403 individuals. Given the application of otolith markers, the assignment to population was not

404 possible in this growth analysis; however, in Table 2, the samples collected in May contained
405 RHS, Baltic and other local spring spawning herring. Thus the spring spawners remaining in the
406 Western Baltic during late spring are probably a mixture of local populations, RHS and
407 populations spawning in the Baltic proper. For genetically assigned herring, Figure 4 panel
408 'May' shows that the fast growing RHS at this time appears in the Kattegat and have left the
409 Western Baltic Sea. Thus the spring spawners remaining in the Western Baltic are a mixture of
410 local populations, slow growing RHS and populations spawning in the Baltic proper.

411 ### *Temporal/Spatial distribution of other herring populations in the WBSS stock*

412 Summarising the occurrence of other herring populations by area and season as analysed from
413 genotyping, otolith analysis, and growth potential, Figure 4 confirms the spatial occurrence of
414 these other spring spawning herring in the study area. Their migration pattern appear
415 somewhat different from the RHS; Table 2 indicates that that the herring in e.g. Lillebælt
416 remain relatively locally during spring and figure 4, lower left panel, indicates these
417 populations are relatively less represented in the outermost feeding areas during summer.

418 ## Discussion

419 ### WBSS population complex

420 Several herring populations were identified using genetic and otolith markers (Table 2) and the
421 statistical analysis of mixed stocks based on both otolith and genetic markers proved to be
422 useful tools for investigating habitat use and migratory behaviour for genetically differentiated
423 populations, even across small spatial scales. We identified several populations resident in the
424 western Baltic and confirmed earlier qualitative descriptions of the herring population complex
425 in the study area (Otterlind 1987). It has been shown previously that herring populations in the
426 case study area may be locally adapted to salinity and that homing to spawning site thus may
427 to some extent be driven by salinity as a selective force (Gaggiotti et al 2009; Limborg et al.,

2012), whereas plasticity in spawning time within location (Clausen et al., 2007; Bekkevold et al., 2007) indicates that specific spawning time is not necessarily a local adaptation (Limborg et al 2012).. Similar behaviour has been described for the herring in the western Atlantic which has been shown to have adaptive reproductive strategies of spawning in response to interannual environmental variability (Melvin et al., 2009).

The key processes affecting population structure can thus be both spawning site fidelity and the migration patterns of the individual herring populations (see Iles and Sinclair, 1982; McQuinn 1997).

Spatial segregation among juveniles from individual spring-spawning populations may facilitate subsequent natal homing (Corten 2001, Gaggiotti et al. 2009) and thus contribute to the observed reproductive isolation between spring-spawning populations from the Skagerrak versus Kattegat and inner Danish Waters (Bekkevold et al., 2005, 2011, Ruzzante et al., 2005). However the migration strategies of the herring populations evidently also affect the population structuring in the WBSS stock.

Rügen herring migration strategy

Results from previous tagging experiments and fishery information indicate a typical migration pattern of RHS between the main spawning grounds around Greifswalder Bodden and surrounding areas to two feeding areas, one main north-westward migration extending to the Kattegat/Skagerrak /North Sea area and one minor eastern migration extending to the area East of Bornholm and the western part of the Hanö Bay (Aro 1989; Biester 1979; ICES 1998; Nielsen et al. 2001; Otterlind, 1987; von Dorrien et al., 2013). This information is, however, only qualitative and indicative (sometimes anecdotal), as well as relatively old and may not reflect the present stock situation and migration patterns. Moreover, local abundances and

452 migrations of both adult and juvenile herring in the Western Baltic Sea throughout the year are
453 not well described.
454 Comparing length at age between herring migrating into the Greifswalder Bodden caught in gill
455 nets over the past 10 years, it was apparent that the timing of spawning seems to depend on
456 age and size as the oldest and fastest growing individuals are migrating into the spawning area
457 first in the season (Figure 2). The difference in growth trajectories between the herring
458 spawning early and late in the season was most pronounced in the older individuals (Figure 2).
459 The identified spawning waves were not described by size of the individual spawner but rather
460 the growth rate of the spawners, where the fastest growing individuals arrived first at the
461 spawning sites confirming earlier results (von Dorrien et al., 2013).
462
463 These first spawners with a high growth potential appear to also be the ones performing the
464 longest summer-feeding migration distance (figure 5c). The growth trajectories of the RHS
465 encountered across the Skagerrak showed some variation for the middle parts of the area,
466 however, the individuals with the steepest growth trajectory was consistently found in the
467 western, outermost parts of the Skagerrak, whereas the slowest growth trajectories were
468 found in the most southern part of the study area (Figure 5). These analyses, thus, suggest a
469 stage dependent migration of RHS during the summer; the individuals with the highest growth
470 rate migrated the furthest out (Figure 5) and apparently only passed through the central and
471 eastern Skagerrak since these 'high growth rate?' individuals were only found in the Skagerrak
472 in June but not in July (Figure 4, lower panels). These results confirm the results found in
473 Clausen et al., 2014, where analysis of spring spawning herring caught in June/July over a 6
474 year period in the North Sea, the Skagerrak and the Kattegat showed the same growth rate
475 dependent spatial distribution. Further, our results indicate that the RHS with a lower growth
476 potential arrives later in the Skagerrak, when the faster growing RHS have left the area; in

Figure 4 (lower panels) the growth curve of RHS lies above the general growth curve in June in the Skagerrak, where it has shifted towards the average growth curve in July.

The Sound (see Figure 1) has been reported as the main overwintering site for RHS (Nielsen et al., 2001). In our study, the RHS were encountered in Kattegat in October and in the W Baltic during November, indicating that the RHS may use those areas for overwintering as well (Table 2), confirming the results in Miethe et al., 2013. However, the majority of the fish encountered in the Western Baltic and Southern Kattegat during autumn are juveniles or young adults (0-3 group makes up more than 50% of the biomass on average; ICES 2013) and thus probably not fully migrating yet. The more elaborate data set based on the long timeseries of survey data on spring spawning herring (Figure 5) showed that the herring following the feeding during summer do appear in the Kattegat and the Sound, confirming earlier reports (Nielsen et al., 2001).

Given that a herring fishery can be observed in the Western Baltic during an entire annual cycle (ICES 2013), it is evident that some herring must occur in the area at all times. Genetic analysis of population affiliation of herring would reveal whether these herring are RHS or Baltic herring; our results from sampling in May indicates the presence of these populations, however, our sampling design did unfortunately not cover the summer in the Western Baltic (Table 2). These individuals typically had a growth pattern different from the migrating parts of the population (Figure 5), however, whether these differences are caused by these non-migrating spring spawners belonging to a local spring spawner population and not RHS, or if this is indeed a mixture of RHS and other spring spawning populations cannot be resolved with the samples available for this study.

Conclusively, the RHS appear to display divergent migration strategies where growth potential is the main structuring factor. The parts of the RHS not performing summer feeding migrations clearly follow a less rapid growth pattern (Figures 5 right panel). The timing of the feeding migration appears to be linked to the growth potential as well given that the RHS with the highest growth potential are the first to spawn (Figure 3); the first to be encountered in the Skagerrak (Figure 4 lower left panel) and then found further out in the Eastern North Sea during the summer (Figure 5c). The RHS spawning later in the spring are arriving later in the Skagerrak and are not migrating as far out of the area (Figure 4, lower panels). The latest spawning RHS may stay in the Western Baltic during the summer, however, the sampling design of our study does not provide conclusive evidence; this would require spatially high resolution monthly samples from the entire study area during a full year. However, our results demonstrate a divergent migration strategy for the RHS population in relation to spawning and feeding migration. It is worth noticing that RHS is the population in the WBSS stock performing the longest migration distances in the area. Thus the further the herring population has to migrate between spawning site and optimal feeding areas, the more evident the migration strategy. Such inter-population diversity indicates a high degree of genetic variance within the population potentially increasing the persistence of the metapopulation through evolutionary time (Palstra and Ruzzante, 2008).

Migration strategies by herring populations in the WBSS stock

The herring populations in our study display to some degree divergent migration strategies; RHS perform summer feeding migrations predominantly driven by growth potential (Figure 5, Clausen et al., 2014), while the spatial distribution of the other spring spawning populations indicates a different migration strategy (Table 2), though still influenced by growth potential (Figure 5). Such spatially divergent distribution of spring spawning populations was also demonstrated in Bekkevold et al., 2011.Herring from the Baltic proper were e.g. observed in

noticeable proportions in the south-western Baltic in autumn, but only in low numbers further north in the Kattegat and Skagerrak. Such divergent migration strategies may contribute to the persistence of the observed genetic differentiation of the herring populations. The processes impacting the critical return migration of adults to the spawning grounds appear to be key factors affecting population persistence through migration strategies (Iles and Sinclair, 1982; McQuinn 1997). Over a single generation, migration is expected to be influenced most by ontogeny, population density, the distribution of habitat values, and in the case of RHS also growth potential (Clausen et al., 2014). Over many generations, variable migratory behaviour, however, should be a key tactic in population persistence (Secor 1999).

Limborg et al. (2012) demonstrated a clear differentiation between herring populations originating from diverse environments in terms of salinity and the genetic analyses suggested that environmental heterogeneity is an important driving force of divergent selection among herring populations. The fact that individuals genetically assigning to RHS are found spawning during winter but in the same spawning site as the spring spawners (Bekkevold et al., 2007), suggests that the structuring factor in terms of spawning is the site characterised by its salinity and not so much the spawning time. Following this, the migration strategy as such is not the only population structuring factor but together with the spawning-site affinity, this behaviour may explain the persistence of a high population differentiation of herring in the area (Iles and Sinclair, 1982; McQuinn 1997; Silva et al., 2013).

Management considerations for the WBSS herring stock

The phenotypic plasticity underlying divergent migration enables flexibility in behaviour, allowing individuals to respond quickly to fluctuations in the environment and maximize fitness (Lundberg 1988; Dingle 1996). Decisions on migration tactics are not necessarily irreversible. In a review of divergent migration tactics, Secor (1999) found that temporal and spatial variations in habitat suitability could cause large variation in fitness for individual migration strategies

552 (Secor 1999). Thus a population displaying divergent migration strategies is capable of
553 adjusting quickly to changes in environment and/or exploitation making the population more
554 resilient. In turn, a stock consisting of several populations with divergent migration strategies
555 and life history strategies would be resilient to changes in the environment and/or exploitation
556 (Schindler et al., 2010). Notably Rügen with its protracted spawning period and variable
557 migration pattern is currently the largest herring population in the area where other herring
558 populations have risen and fallen over time (Alheit and Hagen 1997; Otterlind, 1987). If the
559 population diversity within the population complex is decreased and the dynamics of the
560 populations become more synchronous it may influence population resilence (Hilborn et al.
561 2003; Schindler et al., 2010). A direct consequence of this is that a management of such a
562 diverse stock should be aimed at preserving the population diversity.
563 The herring fishery in the transition area take mixed catches from the two stocks where adult
564 herring in the catches predominantly consist of spring spawners, however originating from
565 several populations. The changed concept of the distribution pattern of the spring spawning
566 herring raises concern in relation to managing the fishery selection of the stock. As the
567 proportion of WBSS is historically lowest in the North Sea, considering management measures
568 without examining the spatial dimension may lead to the suggestion to fish on the apparently
569 healthy stock in the Eastern North Sea while severely restricting fishing in Kattegat, assuming
570 that this would release the WBSS from fishing pressure. This would result in the removal of the
571 largest and fastest growing individuals of spring spawning herring in our study area, which in
572 turn could influence the biocomplexity of the RHS population that is known for its extended
573 spawning period from February to May with the largest individuals arriving first at the
574 spawning ground (Biester 1979, Jørgensen et al., 2005, this paper). A concentration of
575 spawning to a shorter period would increase larval competition and decrease survival with
576 detrimental effects on population productivity. Thus, our results strongly support the notion

577 (e.g. Schindler et al. 2010, Bekkevold et al., 2011) that marine fish management needs to
578 incorporate knowledge about specific population dynamics to allow sustainable exploitation of
579 all stock components. Ignoring such structure, suggested spatial management measures are
580 potentially more devastating to the stock than if no spatial management measures were
581 introduced.
582 When managing a mixture of populations, one must consider the dynamics of the entire stock
583 when estimating recruitment, mortality and other assessment relevant issues, as it is an
584 impossible task to target a single population in a mixed fishery (McQuinn 1997). Thus when
585 managing a stock with a high degree of population diversity, the management needs to be
586 precautionary in order to protect all stock components. In an aggregated management in
587 which a population complex is managed as a single population, extinction of subpopulations
588 could happen before the analyses of aggregated data would indicate a severe population
589 decline (Frank and Brickman 2000).
590 The herring on the western side of the British Isles are essentially a single population (though
591 with many discrete spawning locations) but managed as a series of discrete management units
592 (Geffen et al., 2011). In the North Sea the same approach is adopted, though this stock has
593 been shown to consist of populations with weak genetic differentiation over geographic
594 distance compared to the high differentiation for herring in our study (Mariani et al., 2005;
595 Bierman et al., 2010). The degree of population specific input and application in management
596 is conditional on the ability to assess and manage populations that are defined on biological
597 grounds. Applying a management approach to a stock of mixed populations with low genetic
598 differentiation might be appropriate when those populations cannot be assessed separately
599 (Kell et al., 2009). Such management may be precautionary in the case of the herring
600 populations west of the British Isles and in the North Sea given the low degree of population
601 differentiation. However this is not the case for the mixed fishery on the highly diverse WBSS

602 stock, which also mix with NSAS. A balanced fishery approach could be considered, in which
603 the link between mortality and growth in time and space can be modelled for the individual
604 herring populations (Andersen et al., 2009). Such modelling tools are, however, not yet
605 available and until such specific estimation of the spatio-temporal distribution of herring
606 population constituents can be made, the management of this particular mixed herring stock
607 call for a more precautionary management to account for the greater variety and genetic
608 complexity in the mixed herring catches.

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Tables

Table 1. Samples used in baseline for GSI to determine stock composition in mixed-stock samples. 'Geographic cluster' identifies which of the five genetic baseline clusters individual samples contributed to (see text). Numbers in brackets under 'Sampling location' refer to the locations of samples indicated in Figure 1.

Geographic cluster	Sampling location	Sampling Year	Sampling Month	Peak spawning	Sample size genetic analysis	Latitude	Longitude
1. NE Atlantic	Norwegian Sea	2009	Sep	Mar	31	62.00	11.26
	Iceland	2009	July	Jul	34	63.62	-19.62
	Faroe Islands	2009	November	Aug	30	61.02	-6.38
	Norwegian North Sea (1.1)	2002	Mar	Mar	34	59.25	05.17
2. North Sea	Shetland	2009	Aug	Aug	34	60.35	-2.72
	English Channel	2009	Jan	Nov-Jan	36	50.81	1.57
	Central North Sea	2009	Aug	Aug	30	56.43	0.20
3. W. Baltic	Ringkøbing Fjord (3.1)	2009	Apr	Apr	33	55.97	8.24
	Limfjord (3.2)	2009	Apr	Apr	33	56.60	8.35
	W Skagerrak (3.3)	2003	Nov	Apr	35	58.15	8.27

	S Skagerrak (3.4)	2009	Mar	Apr	36	57.40	11.40
	Kattegat (3.5)	2003	Apr	Apr	23	55.73	11.37
4. RHS	Kiel Canal (4.1)	2011	May	May	20	54.37	10.15
	Schlei estuary (4.2)	2011	May	May	35	54.60	9.76
	Rügen (4.3)	2009	Mar	Mar-Apr	36	54.21	13.62
5. Baltic Sea	Hanö Bay (5.1)	2002	Apr	Apr	24	55.57	15.18
	Gdansk	2009	Mar	Mar	35	54.37	19.67
	Gulf of Riga	2002/2008	May	May-Jun	37	57.83	22.83
	Gulf of Finland	2009	May	May	24	60.40	26.70
	Bothnian Bay	2009	Jun	Jun	33	65.05	24.58

Table 2. Mixed-stock samples genotyped to track RHS migration. Numbers refer to sampling location in Figure 1. The migratory stage of the RHS expected to have been represented in the sample is shown. The numbers of herring assigned to each of the five population clusters, and herring that could not be assigned using the applied accuracy criterion (see text) is given with percentages in brackets. Percentage of herring classified as spring-spawning using otolith microstructure (HM 4) that genetically assigned to RHS is shown.

No.	Area	Lat/Long (median)	N	collection date	capture method	RHS migratory stage	NE Atlan-tic	North Sea	Transi-tion	RHS	Baltic	Un-assign-ed	% HM 4 assignin g to RHS
1	W. Baltic (Arkona)	54°51’47N/ 13°51’47E	47	May 2012	Scientific cruise (*MS Solea*)	Post-spawning migration to feeding areas	2 (4%)		4 (9%)	8 (17%)	21 (45%)	12 (26%)	N.A.[#]
2	W. Baltic (off island of Møn)	54°45N/ 12°30E	40	Nov 2002	Fisherman, netting	Back-migration from feeding areas/wintering	1 (3%)	3 (8%)		35 (88%)		1 (3%)	95%
3		54°58’446N/	44	May 2012	Scientific cruise	Post-spawning	3		3	30	2	6	N.A.[#]

		12°49’918E			(*MS Solea*)	migration to feeding areas	(7%)		(7%)	(68%)	(5%)	14%)	
4	Lübeck Bight	54°24’36N/ 12°11’58E	48	Oct 2009	Scientific cruise (*MS Solea*)ST14	Back-migration from feeding areas/wintering	1 (2%)		2 (4%)	18 (38%)	16 33%)	11 (23%)	28%
5		54°12’04N/ 11°13’36E	48	Oct 2009	Scientific cruise (*MS Solea*)ST2	Back-migration from feeding areas/wintering	2 (4%)		13 (27%)	13 (27%)	10 (21%)	10 (21%)	29%
6		54°15’88N /11°19’76E	73	Feb 2011	Fisherman, netting	Spawning migration		1 (1%)	5 (7%)	44 (60%)	3 (4%)	20 (27%)	64%
7	Kiel Bight (S of Langeland)	54°35’73N/ 10°43’28E	66	Feb 2011	Fisherman, netting	Spawning migration	1 (2%)		8 (12%)	42 (64%)	5 (8%)	10 (15%)	63%
			16	April 2011	Fisherman, netting	Spawning migration/post-spawning			10 (63%)	3 (19%)		3 (19%)	44%

						migration to feeding areas								
8	Lillebælt	55°54’60N/ 10°07’10E	21	April 2011	Fisherman, netting	Spawning migration			13 (62%)	4 (19%)		4 (19%)	38%	
9	Southern Kattegat	55°54’60N/ 11°07’10E	83	April 2011	Fisherman, netting	Spawning migration/post-spawning migration to feeding areas		2 (2%)	6 (7%)	48 (58%)	4 (5%)	23 (28%)	58%	
10	Northern Kattegat (Ålbæk Bight)	57°19’216N/ 10°32’234E	69	April 2011	Fisherman, netting	Spawning migration/post-spawning migration to feeding areas	2 (3%)	10 (14%)	10 (14%)	13 (19%)	2 (3%)	32 (46%)	40%	
11	Central Skagerrak	58°37’12N/ 9°41’24E	38	July 2002	Scientific cruise (*MS Dana*	Summer feeding			4 (11%)	22 (58%)	2 (5%)	10 (26%)	(45%)[†]	

					HERAS)								
12		58°33’36N/ 9°56’59E	38	July 2003	Scientific cruise (*MS Dana HERAS*)	Summer feeding	1 (3%)		2 (6%)	26 (74%)	2 (6%)	4 (11%)	(63%) †
13	Western Skagerrak	57°53’599/ 6°35’398E	81	June 2002	Scientific cruise (*MS Dana HERAS*)	Summer feeding		41 (51%)	4 (5%)	21 (26%)	5 (6%)	10 (12%)	69%
14		57°41’6/ 6°55’1E	84	July 2003	Scientific cruise (*MS Dana HERAS*)	Summer feeding	8 (10%)	27 (32%)	15 (18%)	13 (15%)	1 (1%)	20 (24%)	59%
15		57°51’878N/ 6°44’751E	66	July 2008	Scientific cruise (*MS Dana HERAS*)	Summer feeding		16 (24%)	13 (20%)	21 (32%)	5 (8%)	11 (17%)	69%

#: Hatch month not recorded, †: Sample represents select individuals genetically assigning to RHS based on previous microsatellite based analyses and is thus not representative for the total catch on that location.

Table 3. Self-assignment analysis showing proportions of individuals sampled from each of the five geographic clusters in Table 1 that self-assign back to each of the five genetic clusters (see text). Bold values indicate proportions that assign to their area of origin (collection).

	Assigning to				
Origin	NE Atlantic	North Sea	Western Baltic	RHS	Baltic Sea
1. NE Atlantic	0.97	0.01	0.01	0.01	0.00
2. North Sea	0.01	0.96	0.03	0.00	0.00
3. Western Baltic	0.08	0.16	0.55	0.18	0.04
4. RHS	0	0.03	0.15	0.79	0.03
5. Baltic Sea	0.04	0.01	0.00	0.04	0.92

Table 4. Correspondence between otolith assigned spawning type and assumed spawning type of the genetically assigned mixed stock individuals.

	Genetic assignment					
				Predominantly		
OM assignment				spring	autumn/winter	
of	Assumed spring spawner			spawners	spawners	
spawning type	RHS	BALTIC	NORW.SS	SKAGERRAK	NORTH SEA	UNASS.
spring	238	27	2	91	20	96
autumn	6			8	47	5
winter		1		3	26	1

Table 5 Maximum frequency (mode) among the 8 different maturity stages by month and distance (D) from the Western Baltic spawning grounds (for D see schematic diagram in Material and Methods). Colour scheme (blue to red) indicates maturity stages (ICES 1962) transformed to 0:spent, 1-2:resting 3-5:maturing and 6:spawning.

Sum of mMat	start	Mo										
	1	2	3	4	5	6	7	8	9	10	11	12
Dist_Rugen	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
15		4		2		4	2	4			2	
14	1	4					3			1		
13	3	2					4			2	2	2
12	2	3			4		3				2	2
11	2	3		4		4					2	2
10	2	3	4	3	5		4			2	2	2
9	2	2	2		3							2
8	2	3	2	3		3	3	5		0		2
7	2	2	3	4	3	5	3	4				2
6	2	3	3			3	2	4	3	5		2
5	3	3	3	4	5	4	4	4	4	1		2
4	3	4	3	4	3	3		4	2	1		
3				4	4	4	4	5	1	4	1	
2				2	3	3	4	4	1	2		
1									6			
0	2								5			

Table 6 Model reduction of the von Bertalanffy growth curves for herring collected at collected at the mouth of Greifswalder Bodden during spring (from February through May) from 1998-2005

Model	Number of parameters	Neg.LogLikelyhood	P value
Separate K and L_∞ for month	9	346.1116	
Same K, diff L_∞	6	346.2062	98%
Same K and L_∞ for all	3	382.5954	<0.0001%

Captions

Figure 1. Sampling locations. Circles show local spawning samples used in the genetic baseline (see Table 1 for sample notation and a full list of the applied baseline samples). Stars show mixed-stock samples used in the analysis of RHS migratory patterns (numbers in circles correspond with sample numbers in Table 2). Straight lines indicate ICES management subdivision areas.

Figure 2. ICES rectangle index D, of distance from the Western Baltic spawning grounds off the German coast.

Figure 3. Estimated von Bertalanffy curves with separate levels (L_∞), but common rate (k) for length-at-age data from each month's data. Each data point is plotted with a symbol where the area is proportional to the number of samples it is based on, and hence the weight with which it enters the estimation.

Figure 4. Length at age for the different genetic groups in each month. A common age-length curve is estimated for each month (thick grey line) and for the individual genetic groups (colour thin lines).

Figure 5. Growth potential in herring spring spawners caught on scientific cruises. Numbers are residuals to the von Bertalanffy relation determined length in cm (k=0.508, Linf=28.8025, t0=0). Colour intensity indicates relative growth where red = fast growing, blue = slow growing. Left panel: wintering (Oct-Jan); Middle panel: spring spawning (Feb-May); Right panel: summer feeding (Jun-Sep).

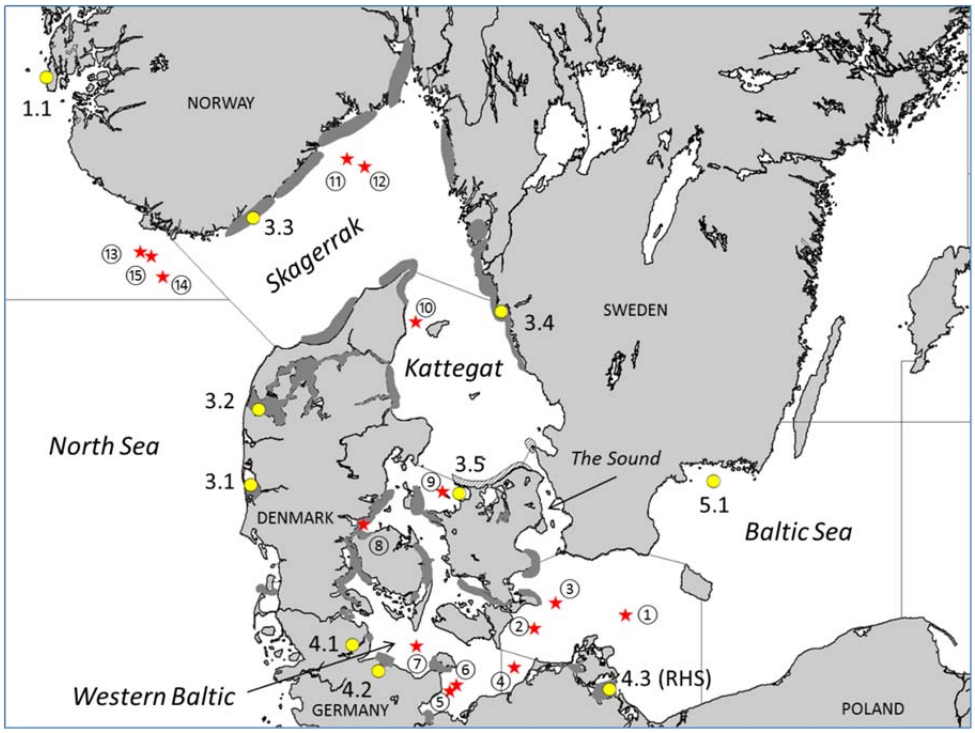

1.1
NORWAY
3.3
Skagerrak
SWEDEN
3.4
Kattegat
3.2
North Sea
3.1
3.5
The Sound
5.1
Baltic Sea
DENMARK
4.1
4.2
4.3 (RHS)
Western Baltic
GERMANY
POLAND

Figure 1.

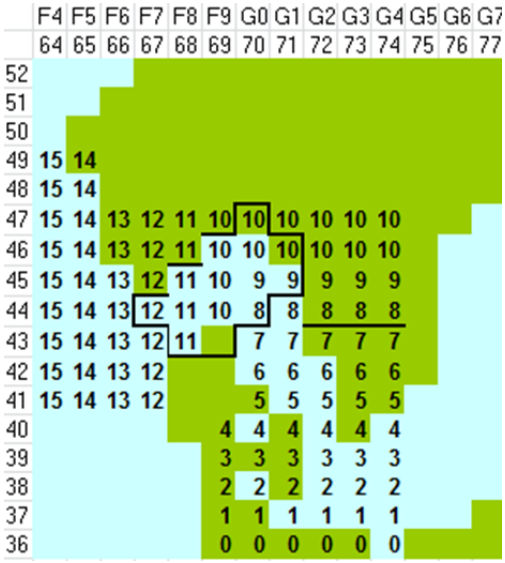

F4 F5 F6 F7 F8 F9 G0 G1 G2 G3 G4 G5 G6 G7
64 65 66 67 68 69 70 71 72 73 74 75 76 77
52
51
50
49 15 14
48 15 14
47 15 14 13 12 11 10 10 10 10 10 10
46 15 14 13 12 11 10 10 10 10 10 10
45 15 14 13 12 11 10 9 9 9 9 9
44 15 14 13 12 11 10 8 8 8 8 8
43 15 14 13 12 11 7 7 7 7 7
42 15 14 13 12 6 6 6 6 6
41 15 14 13 12 5 5 5 5 5
40 4 4 4 4 4 4
39 3 3 3 3 3 3
38 2 2 2 2 2 2
37 1 1 1 1 1 1
36 0 0 0 0 0 0

Figure 2.

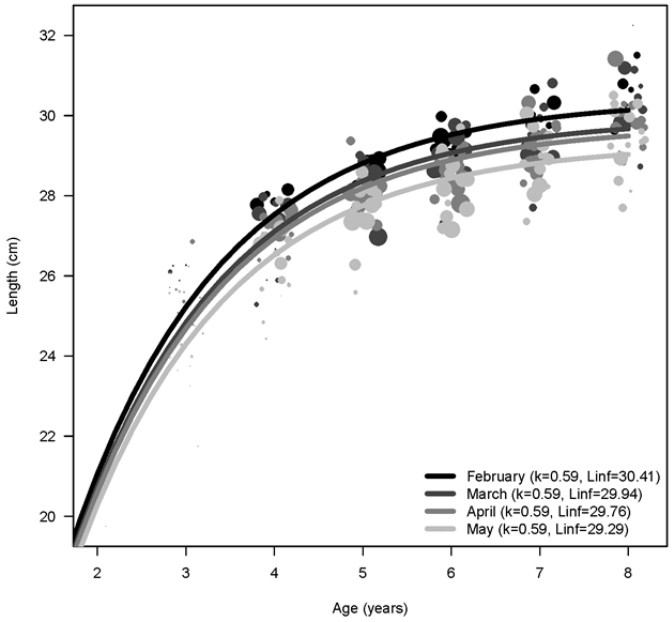

Length (cm)
32
30
28
26
24
22
20
2
3
4
5
6
7
8
Age (years)
February (k=0.59, Linf=30.41)
March (k=0.59, Linf=29.94)
April (k=0.59, Linf=29.76)
May (k=0.59, Linf=29.29)

Figure 3.

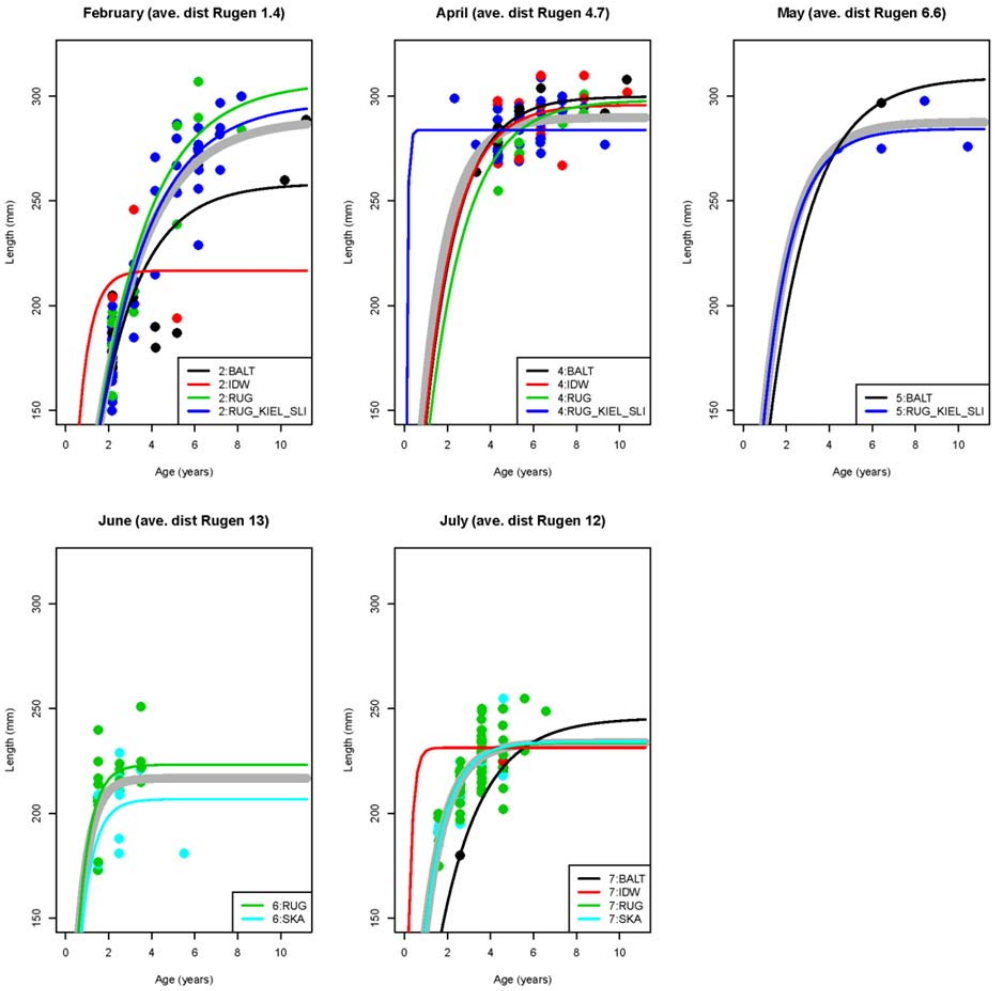

February (ave. dist Rugen 1.4)
April (ave. dist Rugen 4.7)
May (ave. dist Rugen 6.6)
June (ave. dist Rugen 13)
July (ave. dist Rugen 12)
Length (mm)
Age (years)
2:BALT
2:IDW
2:RUG
2:RUG_KIEL_SLI
4:BALT
4:IDW
4:RUG
4:RUG_KIEL_SLI
5:BALT
5:RUG_KIEL_SLI
6:RUG
6:SKA
7:BALT
7:IDW
7:RUG
7:SKA

Figure 4.

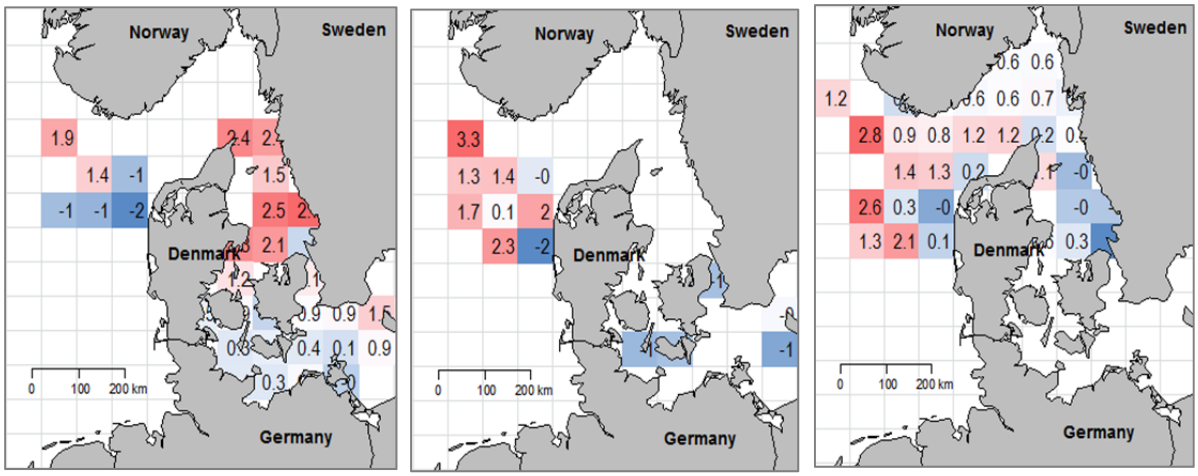
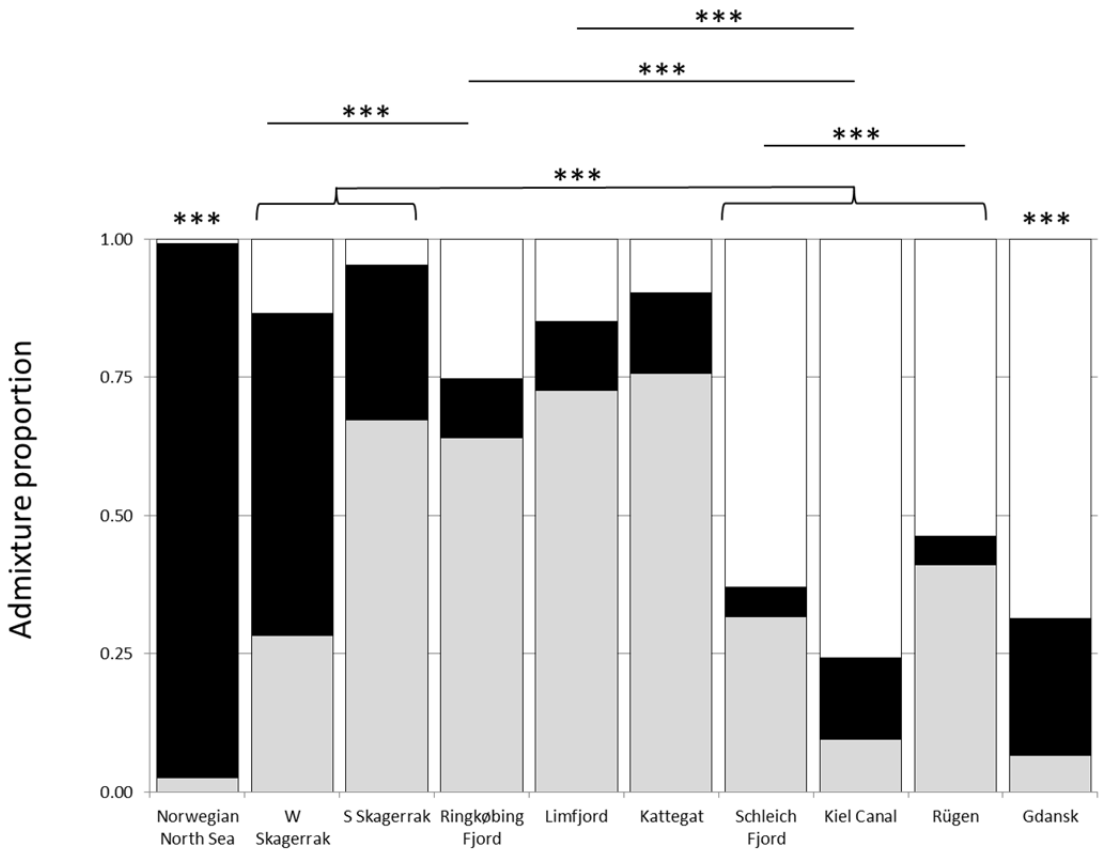

Norway
Sweden
Denmark
Germany
0
100
200 km
1.9
2.4
1.4
-1
1.5
-1
-1
-2
2.5
2.1
0.9
0.9
1.5
0.4
0.1
0.9
0.3
3.3
1.3
1.4
-0
1.7
0.1
2
2.3
-2
-1
0.6
0.6
1.2
0.6
0.6
0.7
2.8
0.9
0.8
1.2
1.2
0.2
1.4
1.3
0.2
-0
2.6
0.3
-0
-0
1.3
2.1
0.1
0.3

Figure 5.

Supplementary Figure



Supplementary figure 1. Sample specific admixture proportions for eight W Baltic samples, one North Sea (Norwegian) and one Baltic (Gdansk), using a model with three clusters. Asterisks above diagram indicate pairwise samples exhibiting highly significant differentiation in exact G tests. All comparisons between either of the two Skagerrak samples and either of the three SW Baltic samples (Schleich, Kiel, RHS) were highly significant (indicated by curly brackets).

Chapter 5: Synthesis and general discussion

This thesis reinforces the fact that herring certainly are not just herring; herring are highly plastic displaying different life history strategies and maintain a complex population structure in the marine environment. This is intriguing in terms of what can be understood of the dynamics controlling a given herring population and its development in terms of persistence over evolutionary timescales under various selective pressures (environmental changes, anthropogenic influences), spatial distribution, and prevailing life history strategy. This complexity also challenge science to find the optimal scientific approaches to disentangle the interrelated regulative factors resulting in the observed complexity inherent in any herring study. Reaching a understanding of the mechanisms behind the dynamics of this highly adaptable fish not only rewards scientific curiosity, but also gives tools for any management of herring resources in a given area and fishery. The Case Study in this thesis condense the origin of herring in an area by means of validated population assignment methods in order to analyse the biocomplexity of the herring population structures and in turn describe the structuring mechanisms of the herring occurrences in an area. **The question asked is which herring are available for a fishery when and where during a year and what may determine any structure of herring occurrences found?**

Which herring makes up the herring soup?

Herring are often characterised based on their spawning time; different spawning time may give rise to different morphometric features (McPherson et al. 2001). Such differences are often used to describe 'races' of herring (Cushing and Bridger 1966; Zijlstra 1969). Overlapping spawning seasons, however, may mislead the interpretation of such differences when used as stock identification method as in the case of using the different signals in the larval otolith microstructure as stock identification method. The gradual change in otolith microstructure from autumn hatch type through late-autumn hatch type to winter hatch type identified in **paper 1** appeared to result in classification of late-hatched autumn spawners as winter hatch types by visual inspection. It was evident that most autumn-hatched herring misclassified using the classic division of hatch seasons were represented by fish hatched late in autumn. Therefore, visual inspection of otolith microstructure may fail to classify individuals hatched in the periods of overlapping spawning seasons if this technique is used as the only stock identification method and **spawning time of the individual herring as inferred from otolith microstructure may thus not be appropriate as the sole stock identification method**. Furthermore, the observation that herring may shift spawning time (**paper 4**; Melvin et al., 2009) makes the spawning time less suitable as a population identification method. However, if used on sufficiently large sample sizes and proper incorporating data on biological parameters, the methodology is applicable to distinguish between herring stocks as currently done – but not populations.

Genetic differences between individuals, stocks and populations are the basis for ascertaining the degree of reproductive isolation, which is the fundamental mechanism structuring difference between these taxonomic groups. The strengths of genetic signals between populations are positively associated with time since divergence of populations (mediated by generation time, with shorter generation time accelerating genetic differentiation) their degree of isolation (reproductive exchange between populations decreasing genetic differences) levels of divergent selection, and negatively associated with genetically effective population sizes (e.g. Hauser and Carvalho 2008). **Paper 2 and 7 collectively identified several herring populations**; three in the Western Baltic, four in the Kattegat and inner Danish waters and one in the Skagerrak (Table 1). The genetic differentiation was most pronounced between the SW Baltic samples (Rügen and two neighbouring populations Schlei and Kiel; light green color in Table 1) and all more northern populations from the Transition area (darker green color in Table 1), suggesting power to differentiate genetically between Rügen and neighboring fjords and other Transition populations with high significance.

STOCK NAME	HERRING POPULATIONS	Western Baltic	Belts and Sound	Kattegat	Skagerrak/ Eastern North Sea
Western Baltic Spring Spawners (WBSS)	Rügen (s,w)	X	X	X	X
	Schlei fjord (s)	X			
	Kiel fjord (s)	X			
	Kattegat (s)		X	X	X
	Lillebælt (s,w)		X	X	X
	Limfjord (s)			X	X
	Ringkøbing fjord (s)			X	X
	Skagerrak (s)			X	X
Norwegian Spring Spawners (NSS)	*				X
North Sea Autumn Spawners (NSAS)	**			X	X

*Table 1: Identified populations in the main herring stocks mixing in the Case study area with their main spawning time annotated in parenthesis, s=spring, w=winter. 'The WBSS are grouped by color indicating differentiation power. X marks recorded occurrence of the population in the area. * Unknown subpopulation structure for NSS (Silva et al., 2013). ** NSAS is composed of Downs, Banks, Buchan and Shetland/Orkney (Dickey-Collas et al., 2010; however NSAS is not resolved into populations in the context of this thesis.*

Genetic differentiation was correlated with geographical distance between spawning sites. However, differentiation was also highly correlated with salinity, indicating that salinity or associated factors were correlated with gene flow among spawning locations. The correlation

between salinity and the genetic differentiation of the herring stocks sampled in the area suggest that the structuring of the herring population is related to the salinity cline throughout the transition area, much along the lines of what has been shown for other fish species in the area (Nielsen et al., 2004; Hansen et al., 2007). Comparing the population differentiation between herring in the Case study area with other adjacent herring complexes (Mariani et al., 2005; Jørgensen et al., 2008), the genetic population structure increased with increasing environmental complexity represented by the salinity cline (Figure 2). A recent study applying SNP assignment of single loci markers under selection in herring investigated the spatial and genomic scales at which herring populations are likely to exhibit adaptation to local environments (Limborg et al., 2011). They identified population differentiation on selected genetic markers related to salinity tolerance (heat shock genes) supporting the results in **paper 2** related to salinity and genetic differentiation.

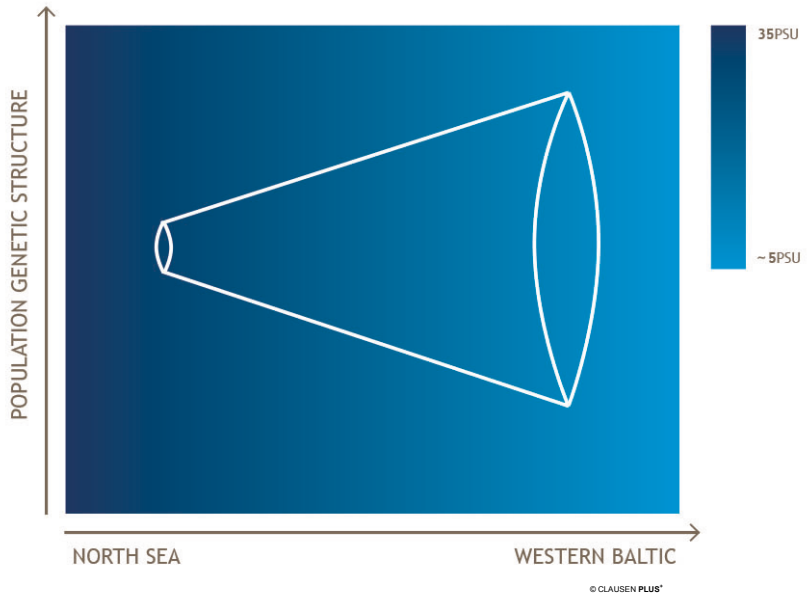


Figure 2.Conceptual figure illustrating the increasing level of biocomplexity with environmental heterogeneity (illustrated by salinity). See text for further explanation.

Through the analysis of markers for the herring populations in the case study it was evident that the applicability of the stock identification methods was different in terms of how operational they were; their accuracy; and their statistical power (**papers 1 – 5**). Having made these multi-marker studies, the way is paved for a critical evaluation of method for stock identification given the validation of the various markers and their applicability. The method choice for future stock identification analysis should depend on the purpose of the analysis. If it is for identification of population diversity in the area, only genetic markers can give the desired resolution at present. However, if the purpose is tracing the management stock of herring in a random sample, otolith analyses has the potential for giving the answer if the assignment of the

local autumn- and winter-spawners can be assigned to the stock in the Transition area and not to the North Sea (as is done currently). The mapping of genetically based differences among herring populations has led to novel procedures for identification and estimation of stock contributions to the mixed-stock fisheries in the transition zone as well as providing baselines for less expensive routine measures of stock identity.

When and where are they – the herring soup?

When migrating, fish populations at times co-occur in areas for a given period (season usually). The extent of mixing with other populations while being part of a mixed stock (in ICES terms, box 1) differs between and even within fish species. Atlantic cod (*Gadus morhua*) maintain population integrity in schools while co-occurring when wintering in Canadian waters (Campana et al., 1999), but appear to mix when co-occurring in Baltic waters (Hüssy et al., 2013). Analyses of mixed-feeding and wintering schools of herring show that population affiliation often varies among herring present in these mixed aggregations (e.g. in the Norwegian Sea: Husebø et al. 2005; the North Sea: Cushing 1967, the Skagerrak: Rosenberg & Palmén 1981, Hulme 1995; west of the British Isles: Brophy & Danilowicz 2002, 2003; and Gulf of St Lawrence: McQuinn 1997a).

Herring migration in the Study area has been characterised as a summer feeding migration from spawning areas distributed in fjords, sils and lagoons to the open waters of Kattegat and in particular the Skagerrak and Eastern parts of the North Sea followed by a return to wintering areas (Biester, 1979; Klinkhart, 1996, vanDeurs and Ramkjær, 2007). Given this seasonality in migration activity, the mixing of herring populations within the Study area must change with season and also location, which indeed is the case. **Paper 3 and 5** conclude that the mixture of herring populations in the Case study area is characterised as being more complex during the summer, when herring migrate to the prevailing feeding grounds in the Kattegat and the Skagerrak areas. As the summerfeeding ends, the herring populations migrates towards wintering areas closer to the coastline, resulting in a more spatially structured herring occurrence in the Case study area. Then the spawning events happen at the particular spawning sites when the timing is appropriate (autumn, winter or spring) depending on the specific life history of the population. In the case of the Rügen herring, the overwintering occurs at a stretch from the Sound towards the spawning grounds around Rügen (Biester, 1979; Klinkhart, 1996; Nielsen et al., 2001; von Dorrien et al., 2013). Thus the complexity of the herring occurrences are geographically scaled with season, summer being the most complex and winter-spring being more structured, where the herring populations appear more close to their respective spawning sites (Figure 3).

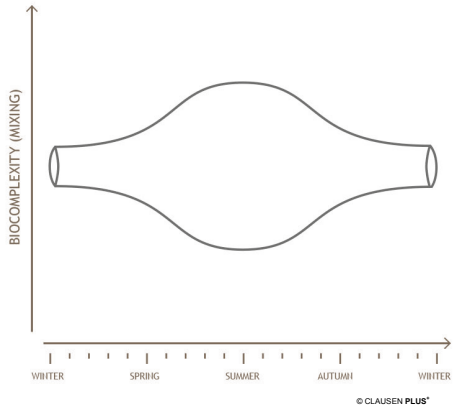


Figure 3.Conceptual figure illustrating the increasing level of biocomplexity during the summer in Northern parts of the study area (Skagerrak, Kattegat). See text for further explanation.

Migrations are assumed to be adaptation to the local environmental conditions for spawning, growth or survival of offspring and maturing individuals. As fecundity of fish increases with body size (Slotte and Fiksen 2000), migrating to better feeding areas will increase the fitness of the migratory individuals unless it is counterbalanced by increases in mortality rates, energy expenditure and delayed maturity as a consequence of the environmental shift (Jonsson & Jonsson, 1993). The spatio-temporal pattern of migrations may vary over time due to e.g. changes in abundance or environmental conditions (Fréon and Misund, 1999; Dingle & Drake, 2007). The migration distance and timing may also vary according to fish age (Harden Jones, 1968; Dingle, 1996; Fréon & Misund, 1999), condition (Slotte, 1999) or other factors such as partial migration (Jonsson & Jonsson, 1993). This may lead to variability in migration distance between years as fish e.g. may migrate further to get to the optimal feeding grounds when they are in better than average condition. Alternatively, migration distance may be a result of density dependent local effects at feeding grounds, with early migrating fish filling an area up to the local carrying capacity leaving the late-comers to find less attractive feeding grounds. The mixing of herring populations (or rather stocks, box 1) during summerfeeding migrations was analysed in **paper 6**, showing a growth-potential related structuring of the summerfeeding migration for the springspawning herring in the Case study area. A clear growth related difference was detected in the Rügen herring; the individuals with the largest size at age were found in the outermost area of the Skagerrak where the feeding conditions are supposed to be optimal (Richardson, 1985, Maar et al., 2013). This supports earlier documentation of a gradient in the Rügen herring feeding migrations; however, these were ascribed to age only (Payne et al., 2009). In **paper 6**, Rügen herring from age 1+ was identified in the Skagerrak, indicating that the onset of the summer-feeding migration is earlier than assumed previously where these migrations were assumed to commence after age 2+ (ICES 2013; Payne et al., 2009). Most importantly, the results demonstrate that the distance of the summer feeding migration is determined by factors related to growth potential of the individual herring, which is illustrated in Figure 4.

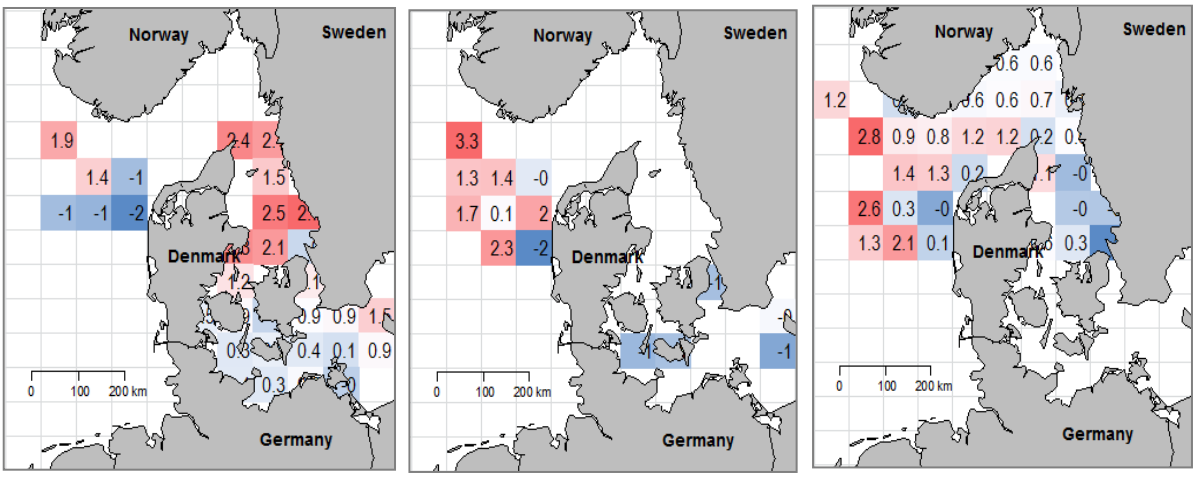


*Figure 4 (from **Paper 7**). Growth potential in herring spring spawners caught on scientific cruises. Numbers are residuals to the von Bertalanffy relation for Rügen herring determined length in cm (k=0.508, Linf=28.8025, t0=0). Colour intensity indicates relative growth where red = fast growing, blue = slow growing. Left panel: wintering (Oct-Jan); Middle panel: spring spawning (Feb-May); Right panel: summer feeding (Jun-Sep).*

Thus in terms of 'when and where' of the herring populations making up the herring stock(s) in the Case study area a clear seasonal gradient in terms of mixing is evident, however, within the most mixed period, structure is found related to the growth potential of the herring within the populations as illustrated in Figure 5.

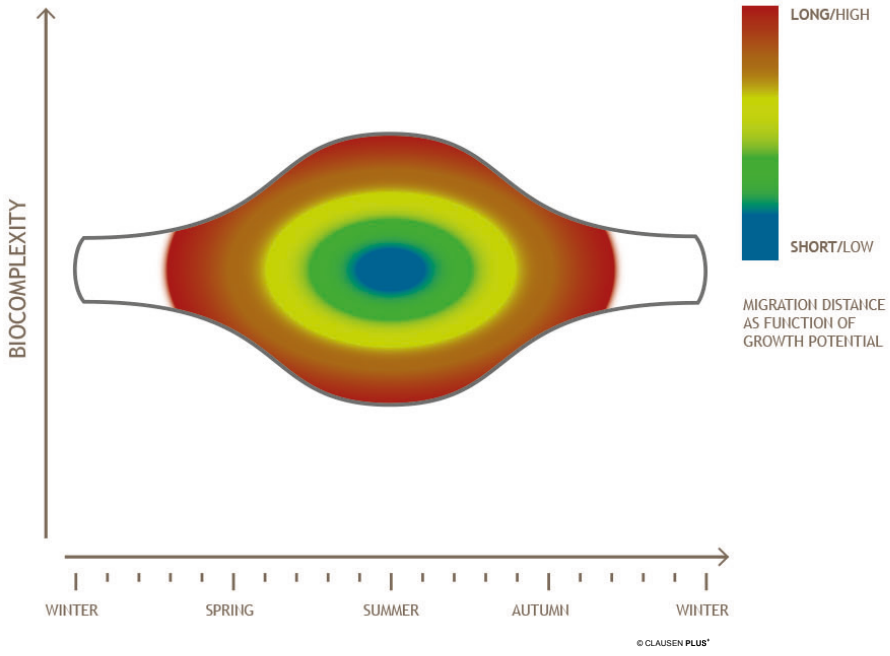


Figure 5.Conceptual figure illustrating the increasing level of biocomplexity during the summer in Northern parts of the study area (Skagerrak, Kattegat). The colors represent growth potential (low:blue; high:red) which is a structuring factor during the highly mixed period in summer.

The demonstrated complex pattern of intraspecific diversity of the herring populations along the salinity cline must be upheld because of the existence of structuring factors ensuring a high degree of population integrity in terms of spawning events. These individual populations give rise to production of offspring with different genetic composition, exhibiting variation in both behaviour and life history (**paper 3, 5, and 6**).

What structures the biocomplexity; the herring soup 'stock'?

For herring in the transition zone, temporally stable differentiation in highly polymorphic microsatellite DNA markers among spawning locations were identified despite the fact that individuals migrate freely across it (**paper 2**). Looking further into patterns of genetic differentiation in a relatively small geographical part in the Case study area, **paper 4** identified a winter spawning stock in Lillebælt that was genetically relatively strongly divergent from sympatric spring spawning stocks, whereas a winter spawning stock from Rügen in the Western Baltic did not differ from the dominating spring spawning stock. However, these investigations were done applying microsatellite DNA markers which may have limitations in assignment in stocks where gene flow is relatively large and/or where effective population sizes are large. For instance, these markers are based on differentiation developed through genetic drift and may thus not be powerful enough to immediately detect if a fraction of a stock 'switch' spawning time and found a new reproductively isolated stock, as may have been the case in the Rügen winter spawners (**paper 4**). These results do not demonstrate a role for direct linkage between microsatellite DNA loci and traits under selection (but see Gaggiotti et al., 2009), but rather that populations experiencing dissimilar salinity conditions on spawning locations follow different evolutionary trajectories. However, applying SNPs Limborg et al. (2011) demonstrated the applicability of this type of markers under selection to distinguish herring origin on a regional level, with a higher statistical power than microsatellites. They demonstrated a clear differentiation between herring populations originating from diverse environments in terms of salinity and the genetic analyses suggested that environmental heterogeneity is an important driving force of divergent selection among herring populations. The fact that individuals genetically assigning to spring spawning Rügen herring are found spawning during winter but in the same spawning site as the spring spawners (**paper 4**), suggests that the structuring factor in terms of spawning is the site characterised by its salinity and not so much the spawning time.

Thus a clear structuring factor in herring population integrity is the salinity cline throughout the case study area. This area has previously been described as a 'Hybridzone' for other fish species like cod (Nielsen et al., 2003), turbot (Nielsen et al., 2004) and European flounder (Hemmer Hansen et al., 2007), where the genetic differentiation between populations in a transect from the saline North Sea to the brackish Baltic proper best were described by a hybridzone between two highly differentiated populations in either end of the transect. These studies were done on microsattelite loci and thus perhaps not with the same high levels of

assignment power for evaluating hypotheses of fish origin as SNP loci analysis have. Using such techniques in more recent studies (**paper 7**, Nielsen et al., 2012; Limborg et al., 2012) however, herring show significant differentiated population structure in this geographical area, supporting the hypothesis that a structuring factor for herring populations in this area in relation to spawning is indeed the salinity at the spawning site. This is somewhat divergent from previous assumptions where herring 'races' were assigned to their time of spawning (Cushing 1967) which probably still can be used to differentiate between larger population complexes across larger geographical areas. However the plasticity in spawning time (**paper 1 and 4**) and apparent salinity associated genetic markers (**paper 2**, Limborg et al., 2012) indicate that herring populations in the case study area are locally adapted to salinity and that they home to spawning site but necessarily not to spawning time. Herring in the western Atlantic have been shown to have adaptive reproductive strategies of spawning in response to interannual environmental variability (Melvin et al., 2009). This supports the conclusion that spawning site is what structures the herring populations in the case study area in terms of reproductive strategy and not the spawning time.

The processes impacting the critical return migration of adults to the spawning grounds appear to be key factors affecting population persistence (see Iles and Sinclair, 1982; McQuinn 1997). Spatial segregation among juveniles from individual spring-spawning populations may facilitate subsequent natal homing (Corten 2001, Gaggiotti et al. 2009) and thus contribute to the relatively large reproductive isolation between populations from the Skagerrak versus Kattegat and inner Danish Waters (**paper 2 and 7**). Migration is frequently described as an adaptive, long-distance movement occurring predictably during a defined time-cycle (e.g. annually or a full life-cycle). These movements take advantage of spatial and temporal differences in the distribution of resources (being food, spawning habitat availability, shelter for predators, etc.), and thus increase the fitness of the migrants (Harden Jones 1976; Smith 1985; Chapman et al., 2012). For such behaviour to evolve, the benefits of using two or more different areas during a defined time-cycle must outweigh the costs of the migration. The extent and timing of fish migrations are generally both consistent and predictable within a given population, suggesting an evolutionary advantage (Harden Jones, 1965; Smith, 1985; Campana et al., 1999), though variation in migration pattern may occur within populations (Slotte and Fiksen 2000). In Western Atlantic herring, the spatial distribution of the various life-history stages has a gradient in terms of mixing; the spawning grounds are fixed, whereas the overwintering and in particular summer feeding areas and spawning time may shift following environmental changes (Melvin et al., 2009; Sinclair and Iles 1985). This is somewhat similar to what is seen in the herring populations in the case study; the migration strategy is different between and even within populations (Rügen, **paper 7**); however, they home to spawning site. This migrating behavior is the main structuring factor of the herring populations in the case study area; the summerfeeding migrations are individually determined by growth potential and food availability; the back-migration to wintering areas diverge between populations and the spawning migrations are

determined by local adaptation to salinity; they return to spawning site but not necessarily to spawning time.

The herring populations in the case study do to some degree display divergent migration strategies; they perform summer feeding migrations predominantly driven by growth potential (Figures 4 and 5; **papers 6 and 7**) spatially structuring the herring populations during summer. However, the results in **paper 2 and 7** indicate that the locally adapted herring populations in Kattegat may not migrate the long distance to the outer Skagerrak but remain relatively locally during summer. Such divergent migration strategies may contribute to the persistence of the observed genetic differentiation of the herring populations (**papers 2:5, 7**), however even within the larger Rügen population, divergent migration patterns are evident (**paper 7**). Following this, the migration strategy as such is not the only population structuring factor but together with the spawning-site affinity, this behaviour may explain the persistence of a high population differentiation of herring in the area (Iles and Sinclair, 1982; McQuinn 1997; Silva et al., 2013).

It is worth noticing that Rügen herring which have divergent migration strategies is the population performing the longest migration distances in the area. **Paper 7** confirms a growth potential driven migration strategy from spawning towards feeding for spring spawning herring in the study are. The identified spawning waves were not described by size of the individual spawner but rather the growth rate of the spawners, where the fastest growing individuals arrived first at the spawning sites confirming earlier results (von Dorrien et al., 2013). These first spawners were also the ones performing the longest summerfeeding migration distance. Thus the further the herring population has to migrate between spawning site and optimal feeding areas, the more evident the migration strategy. Such inter-population diversity indicates a high degree of genetic variance within the population potentially increasing the persistence of the population through evolutionary time (Palstra and Ruzzante, 2008). Notably Rügen is currently the largest herring population in the area where other herring populations have risen and fallen over time (Alheit and Hagen 1997; Otterlind, 1987).

Conclusions and Perspectives

Thus, which herring are available for a fishery when and where during a year and what may determine any structure of herring occurrences found? With offset in the Case study, the answer in short is: Many genetically different herring populations are available for a fishery; their occurrence is structured by divergent migration strategies driven primarily by growth potential and the persistence of a genetic population differentiation is linked to the environmental heterogeneity in terms of salinity facilitating homing to spawning site (but not necessarily spawning time). Figure 6 summarise the main structuring factors of herring populations in the Case study.

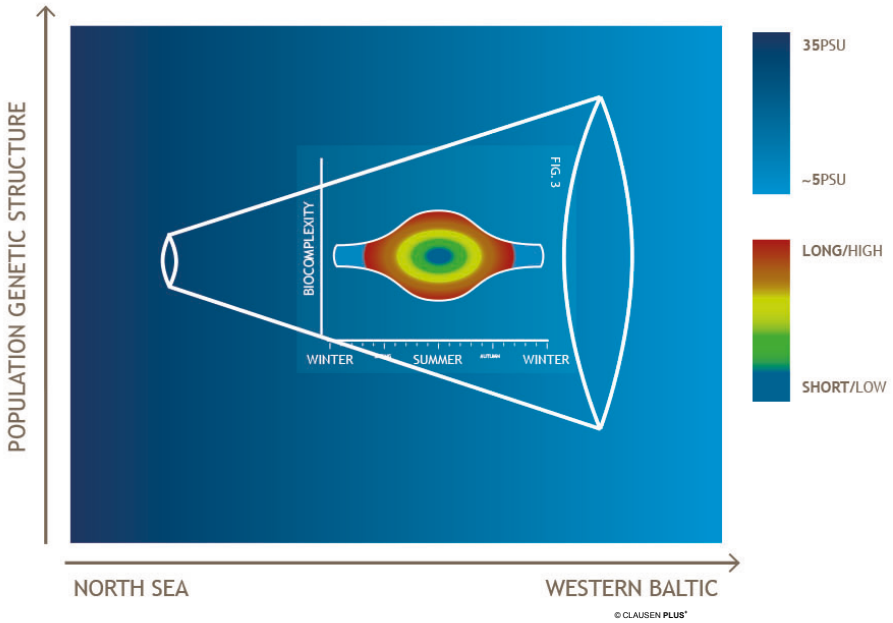


Figure 6. Summarizing the biocomplexity of herring in the case study and the structuring factors: environmental heterogeneity (expressed as salinity) and a growth potential driven divergent migration pattern. See text for further explanation.

The results in this present thesis thus contribute to the understanding of the dynamics of the herring populations in the mixed pool of herring in the transition area between the North Sea and the Baltic. Many questions, though, lie ahead; how do the herring manage to maintain the migration route from the summerfeeding to the population specific wintering area after having been mixed with other populations? And why do young Rügen herring with a high growth potential perform apparent unfavorable migrations in their beginning of life, loosing condition while migrating to the outer Skagerrak? Future research could advantageously combine analysis of growth (from e.g. otolith microstructure) with SNP marker analysis to disentangle the specific life history strategies of the herring within and between populations and through this achieve even more detailed insight of the structuring factors of the large herring soup in the area.

The diversity of the herring populations in terms of spawning site, time and migration strategy found in the present thesis underlines the plasticity of the herring life history strategies (Geffen, 2009). Seen as a stock, the herring in the Western Baltic, the Kattegat and the Skagerrak constitutes a pool of herring with high genetic variability effectively resulting in a stock with ability to sustaining itself through time (Hilborn et al. 2003; Schindler et al., 2010). A mixed fishery targetting specific population, or even parts of populations, within stock may lead to a reduction in the capacity of the stock to withstand climate variability and change; i.e. the resilience of the stock (Planque at al., 2010; Schindler et al., 2010).

When managing a mixture of populations, one must consider the dynamics of the entire stock when estimating recruitment, mortality and other assessment relevant issues, as it is an impossible task of targeting a single population in a mixed fishery (McQuinn 1997). Thus when managing a stock with a high degree of population diversity, the management needs to be precautionary in order to protect all stock components. In an aggregated management in which a population complex is managed as a single population, extinction of subpopulations would be possible before the analyses of aggregated data would indicate a population decline (Frank and Brickman 2000).

The herring on the western side of the British Isles are essentially a single population (though with many discrete spawning locations) but managed as a series of discrete management units (Geffen et al., 2011). In the North Sea the same approach is adopted, though this stock has been shown to consist of populations with weak genetic differentiation over geographic distance compared to the high differentiation for herring in this case study (Mariani et al., 2005; Bierman et al., 2010). The degree of population specific input and application in management is conditional on the ability to assess and manage populations that are defined on biological grounds. Applying a management approach to a stock of mixed populations with low genetic differentiation might be appropriate when those populations cannot be assessed separately (Kell et al., 2009). Such management may be precautionary in the case of the herring populations west of the British Isles and in the North Sea given the low degree of population differentiation. However in the case study, there is not just mixing of spawner types with low genetic differentiation, but mixing of spawner types with high genetic differentiation. To exploit this stock in the same manner as the western British Isles herring or the autumn spawning North Sea herring would require a higher resolution of monitoring. This is probably not realistic. Thus the management of this particular mixed herring stock call for a more precautionary management to account for the greater variety and genetic mix in the mixed herring catches.

As the understanding of the dynamics of herring populations increase based on among others the work in the present thesis, the concept of the management ‘stock’ is challenged. The combined disciplines of high resolution genetic marker analysis and analysis of biological parameters such as growth can be used for refinement of fishery and resource monitoring approaches and the optimal sampling design for confirmatory analysis and possibly stock composition analysis. Such insight would aid a sustainable aggregated management of a fishery on a mixed (herring) stock. It would facilitate managing according to the evolutionary population concept, protecting the weaker populations from over harvesting in a mixed fishery. This is required to maintain the diversity and in turn the resilience of the stock to a fishery.

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When you study herring, there are no wrong answers
A.J. Geffen at ‘Linking herring’ Symposium, 2009

Fish are not just fish. Differences within marine fish species in terms of morphology, behaviour, life history and certainly also genetic differentiation have been shown for an impressive number of species, including herring (*Clupea harengus*). This PhD thesis explore the population complexity of the herring stock called the Western Baltic Spring Spawning herring; localized in the transition area between the North Sea and the Baltic. The results in this present thesis contribute to the understanding of the dynamics of the herring populations in the mixed pool of herring in the transition area between the North Sea and the Baltic. Such insight will aid a sustainable aggregated management of a fishery on a mixed herring stock.

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